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An Overview of Ultrasound: Theory, Measurement, Medical Applications, and Biological Effects

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An Overview of Ultrasound: Theory, Measurement, Medical Applications, and Biological Effects

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WHO Collaborating Centers for:
- Standardization of Protection Against Nonionizing Radiations
- Training and General Tasks in Radiation Medicine
- Nuclear Medicine

July 1982

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Food and Drug Administration
Bureau of Radiological Health
Rockville, Maryland 20857

FOREWORD

The Bureau of Radiological Health develops and carries out a national program to control unnecessary human exposure to potentially hazardous ionizing and nonionizing radiations and to ensure the safe, efficacious use of such radiations. The Bureau publishes the results of its work in scientific journals and in its own technical reports.

These reports provide a mechanism for disseminating results of Bureau and contractor projects. They are distributed to Federal, State, and local governments; industry; hospitals; the medical profession; educators; researchers; libraries; professional and trade organizations; the press; and others. The reports are sold by the Government Printing Office and/or the National Technical Information Service.

The Bureau also makes its technical reports available to the World Health Organization. Under a memorandum of agreement between WHO and the Department of Health and Human Services, three WHO Collaborating Centers have been established within the Bureau of Radiological Health, FDA:

WHO Collaborating Center for Standardization of Protection Against Nonionizing Radiations;

WHO Collaborating Center for Training and General Tasks in Radiation Medicine; and

WHO Collaborating Center for Nuclear Medicine.

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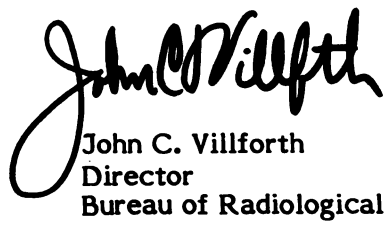


John C. Villforth
Director
Bureau of Radiological Health

PREFACE

Scientific intelligence and program support in the physical and biological sciences are provided to the Bureau of Radiological Health by the Division of Electronic Products and the Division of Biological Effects. Among the responsibilities of the Division of Electronic Products is the physical evaluation and characterization of radiation emissions from electronic products. The Division of Biological Effects plans, conducts, supports, and reviews epidemiologic and experimental studies on the health implications of exposure to electromagnetic and acoustic energy as a basis for risk assessment.

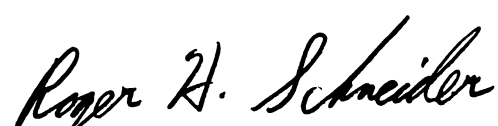
During the past decade, medical ultrasound has proliferated dramatically to become a valuable tool for diagnostic, therapeutic, and surgical use. The Bureau of Radiological Health foresaw this development and brought it to the attention of the Surgeon General and the Commissioner of Food and Drugs. Extensive interactions have taken place with other agencies, including the National Science Foundation and the National Institutes of Health, and with professional and industry groups including the American Institute of Ultrasound in Medicine, the Acoustical Society of America, the National Council on Radiation Protection and Measurements, and the National Electronic Manufacturers Association, with whom Bureau staff have collaborated in the development of a standard.

Out of these interactions and as a result of activities on the part of responsible scientists and public health officials in Canada, Great Britain, and other countries, basic physical and biological research programs developed, conducted both in the laboratories of the Bureau of Radiological Health and in major research centers throughout the world. These have focused on the characterization and measurement of acoustic fields, and on the biological effects and health implications of exposure to these fields. This report brings together much of the information that was developed through these scientific programs.

Drs. Stewart and Stratmeyer, editors of this report, have been responsible for the development and implementation of Bureau programs in the ultrasound area. They and their professional colleagues within the Bureau have assembled this report, which presents the current state of knowledge. Much of this information was incorporated into the WHO (Geneva) report, "Environmental Health Criteria Document on Ultrasound," now in the final stages of revision. BRH staff have been heavily involved in the development of the WHO document, as well as in the development of an Ultrasound Chapter for a Manual on Nonionizing Radiation, being prepared by the European Office of the World Health Organization.

It is our hope that this report will benefit those who work in the field of ultrasound: the manufacturers of instrumentation; the health professionals who have developed the many valuable applications of this modality; and the scientists who continue to improve our understanding of the interactions and mechanisms of ultrasound with biological tissue.

Moris L. Shore, Ph.D.
Director
Division of Biological Effects



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ABSTRACT

Stewart, H.F. and M.E. Stratmeyer, Editors. An Overview of Ultrasound: Theory, Measurement, Medical Applications, and Biological Effects. HHS Publication FDA 82-8190 (July 1982) (pp. 134).

This document examines medical ultrasound in the United States. The first part of this report provides background information on characteristics and interaction of ultrasound with matter, measurement of ultrasound fields, and medical applications of ultrasound.

The second part of this report emphasizes the potential biological effects of ultrasound in humans and experimental animals and the possible mechanisms for producing these effects. The potential risk to public health is evaluated and recommendations are made for the safe use of this modality. Recommendations are also made for further research to help clarify the potential biological risk associated with this rapidly growing modality.

The opinions and statements contained in this report may not necessarily represent the views or the stated policy of the World Health Organization (WHO). The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services (HHS) or the World Health Organization.

AN OVERVIEW OF ULTRASOUND: THEORY, MEASUREMENT, MEDICAL APPLICATIONS, AND BIOLOGICAL EFFECTS

1. INTRODUCTION

Ultrasound, pressure waves propagated in a medium at frequencies which are above the normal ranges of human audibility, can be propagated in liquid, solid, and gaseous media. The highest audible frequencies for humans are about 16-18 kHz, and for some animals even higher. Acoustic energy is usually considered as one of the nonionizing forms of radiation because it does not normally cause the production of ions in the irradiated material.

Ultrasound has found increasing application in recent years. Many applications of ultrasound involve human exposure either incidentally or as an essential part of a procedure, as in medical applications. Such exposures inevitably raise questions concerning risk to human health. This document will provide some general information about physical characteristics of ultrasound and measurement methods, discuss some of the medical applications of ultrasound, review the reported effects of ultrasound on exposed biological systems, and evaluate the health risk and make recommendations for safe use and for future research given the present state of knowledge.

Section 2 provides background on the characteristics and interaction aspects of ultrasound fields. Section 3 provides information on the measurement of ultrasound fields. Section 4 provides a brief overview of the applications and use of ultrasound for various applications. Section 5 reviews the biological effects data. On the basis of information in the previous sections of the document, summary and conclusions are given in Section 6. Section 7 lists recommendations for further research. Depending on the reader's background, the reader may prefer to go directly to the section of interest.

2. CHARACTERISTICS AND INTERACTIONS OF ULTRASOUND FIELDS

Ultrasonic energy is transported by mechanical vibrations at frequencies above the upper limit of the frequency range for human audibility (generally accepted as about 16 kHz). The ultrasound consists of a propagating periodic disturbance in the elastic medium, causing the particles of the medium to vibrate about their rest (equilibrium) positions. Here the term "particle" refers to a differential element of volume in the medium. The vibratory motion of the particles is essential to energy propagation; unlike electromagnetic radiation, acoustic energy cannot be transmitted through a vacuum. The transmission through the medium is strongly dependent on the ultrasound frequency and the state of the medium--gas, liquid, or solid. For example, the intensity of an ultrasound beam transmitted from one region of tissue to another is greatly reduced if a layer of gas intervenes.

The passage of a sound wave through a medium can be characterized by several parameters which are associated with the movement of particles in the medium. Some of these parameters are acoustic pressure (p), particle velocity (v), acceleration (a), and displacement (s).

The acoustic pressure (p) is the difference between the total pressure and the static (equilibrium) pressure as the wave propagates through a given point in the medium. Thus, p is positive during the compression part of the wave and negative during the rarefaction part. The particle velocity (v) is the velocity of the vibrating particles moving about their equilibrium positions. This should not be confused with the speed of the sound (c); the latter is the speed with which the wave passes through the medium, even though the individual particles of the medium vibrate only about their normal position with no bulk movement of matter. The speed of the sound (c) is a constant which depends on the physical properties of the medium and is discussed further in section 2.2. The displacement (s) is the change in position of a particle from its equilibrium position. The density (ρ) changes as the medium is compressed or expanded; the fractional density change (ρ/ρ_o-1) is termed the condensation.

The instantaneous acoustic intensity (I) of a sound wave is defined as the time rate of flow of energy through a unit area. For continuous waves the unqualified word "intensity" is commonly understood to mean the temporal average of the instantaneous intensity. The relationship between intensity and the various particle parameters (velocity, displacement, and acoustic pressure) may be of importance for some biological effects reported in the literature (Child et al., 1981). These relationship are given in Appendix I.

Pulsed diagnostic sources have short and widely spaced pulses with high temporal peak values compared to continuous waves having the same temporal average intensity. During a pulse from a diagnostic ultrasound pulse-echo machine, the particle velocity and displacement in an aqueous medium have values comparable to those for continuous wave (cw) therapeutic ultrasound; the major difference is the time duration. The particles are exposed to these ultrasonic fields for extremely short times (typically, a 1 microsecond pulse spaced at intervals of 1 millisecond) in diagnostic pulse-echo applications and continuously in therapeutic exposure. The peak acoustic pressure to which the particles are subjected during a pulse from a diagnostic ultrasound pulse-echo machine can be about one order of magnitude larger than that in therapy. The particle parameters are also a function of frequency; for example, acceleration increases with frequency while displacement decreases. Therefore, it is important that the frequency of the ultrasound be known. Likewise it is important to know the temporal peak intensity as well as the time (or temporal) average intensity for pulsed ultrasound. For example, pulse-echo devices used in medical diagnosis have duty factors on the order of 1:1000. If exposure intensities are expressed in time average values, the peak values of intensity are 1000 times higher and the particle parameters are higher by a factor equal to the square root of 1000. These are

important considerations when comparing pulsed or continuous waves for biological effects. Comparison of the temporal average values only may be inadequate.

An important difference between ionizing radiation and ultrasound should be noted here. To increase the intensity of a beam of x rays of a given spectral distribution, one increases the photon flux. The energy of individual photons remains unchanged. Therefore the interaction mechanism for each photon remains the same but the number of interactions increases because of the increased number of photons. To increase the intensity in a beam of ultrasound of fixed frequency, one increases the amplitude of the particle parameters (displacement, velocity, acceleration), to obtain a higher energy flux per unit area. Therefore, because of the change in the magnitude of the particle parameters some mechanisms of interaction with matter may become more important at different intensities. For example, in a study of the effects of ultrasound on the reproductive integrity of mammalian cells cultured in vitro, Martins (1971) concluded that for a given frequency, the effectiveness in causing cell death probably depends on the amplitude of the waves for a given cell line.

2.1 INTENSITY DISTRIBUTION IN ULTRASOUND FIELDS

Many of the ultrasound fields encountered in actual exposure of humans, or in related biological experimentation, may be quite complex, but most can be considered to be somewhere between two extreme types: the plane progressive wave field and the standing wave field. Appendix I gives the relationships between the parameters discussed in section 2 for the plane progressive wave field.

2.1.1 Plane Wave Progressive Fields

The field radiated by a vibrating piston has been of longstanding theoretical interest, principally because of its usefulness in modeling the field of the transducer. A historical review and analysis of previous studies and a theoretical generalization to account for nonuniform surface vibrations has been given recently (Harris, 1981a, 1981b). Much of the discussion that follows is based on this model.

The ultrasonic field produced by a transducer obeys all the physical laws of wave phenomena. It can be thought of as being produced by many small point sources (Huygens sources) making up the transducer face and thus producing a characteristic interference pattern at any point in the field.

As ultrasound leaves the transducer, there is a zone where the overall beam width remains relatively constant although there are many variations in intensity within the field itself, both across and along the beam axis. This is followed by a zone where the beam diverges and the intensity variations become smoother. For continuous wave operation the beam is generally thought of as being made up of these two regions, the near field (or Fresnel region) and the far field (or Fraunhofer region). A numerical analysis of the near field of a vibrating piston has been described in the literature (Zemanek, 1971). For a given radius of the transducer, the near field is quite complex, exhibiting more maxima and minima as the wavelength of the ultrasound becomes smaller. In the far field, the acoustic intensity is proportional to the square of the acoustic pressure (Appendix I). The directivity of the beam in the far field is determined by diffraction effects in the same way that a beam of light is affected by aperture size. The higher the frequency of ultrasound produced for a given transducer size the more directional is the ultrasound; for a constant frequency, a large diameter beam is more directional than a small one. Equation 2.1 presents the formula for determining the angle of divergence, θ, in the far field as shown in Figure 2.1 (Kinsler & Frey, 1962).

$$\sin \theta = 0.61(\lambda/r) \qquad \text{Eq. 2.1}$$

where,

r = radius of the vibrating piston radiator
λ = wavelength of sound in the propagating medium

Therapeutic transducers with beam areas of less than 5 cm^2 have been regarded by some as being unacceptable because of beam divergence (Lehmann, 1965). Figure 2.1 conceptually illustrates the far field and near field zones and the intensity distribution along the central axis of the beam.

Schematic Representation of Ultrasound Beam and Axial Intensity Profile

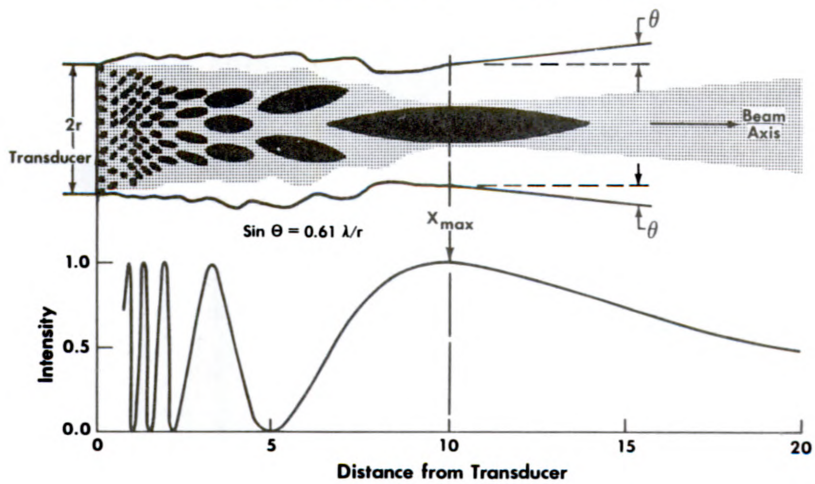

Figure 2.1. Conceptual illustration of the intensity distribution along the central axis at a vibrating piston radiator. X_{max} is the distance at which the maximum intensity occurs.

For a beam produced by a plane circular piston source, the point of maximum intensity lies on the beam axis. The relationship which describes the intensity distribution along the beam axis of a continuous wave field for this type of source is (Kinsler & Frey, 1962; Wells, 1977):

$$I_x = I_o \sin^2 (((r^2 + x^2)^{1/2} - x)(\pi/\lambda)) \qquad \text{Eq. 2.2}$$

where I_o = spatial maximum intensity

I_x = intensity at a distance x from the transducer with a radius r.

Equation 2.2 is plotted in the lower part of Figure 2.1 for a piston diameter of 2.54 cm radiating into water and having an ultrasonic frequency of 1 MHz (i.e. λ = 0.15 cm). The distance from the transducer face to the last axial maximum (x_{max}) is given by

$$x_{max} = (4r^2 - \lambda^2)/4\lambda \qquad \text{Eq. 2.3}$$

If $r \gg \lambda$ then x_{max} is given approximately by r^2/λ.

In practice, the intensity distribution along the axis of an ultrasound transducer will be different from the theoretical behavior described above. In particular, the various maxima observed on the axis may not all have the same value. The largest of the maxima is referred to as the spatial peak intensity in the field. For exposures in experimental studies,

spatial peak (SP) intensity may refer to the local maximum within the specific exposed region. It is also possible to define a spatial average (SA) intensity as the ratio of the power to the beam cross sectional area in the plane of interest. The definition of these terms is given in Appendix II.

For a theoretical plane circular piston, it can be shown that the spatial maximum intensity is 4 times greater than the spatial average intensity (Zemanek, 1971; Nyborg, 1977). In actual practice, this ratio ranges from 2 to 6, although higher values may be encountered depending on such factors as the nature of the piezoelectric crystal used and how it is mounted in the applicator housing (Stewart et al., 1982).

2.1.2 Plane Standing Wave Fields

The standing wave field occurs when two waves are propagated simultaneously in the same medium but in different directions. The resultant waveform at any instant is obtained by adding the wave pressures at each point. It is characterized by a stationary pattern of minimum and maximum pressure amplitudes called "nodes" and "antinodes," respectively. Thus standing waves can give rise to regions of enhanced amplitude. This situation can arise when continuous ultrasound is propagated into a confined space, so that the ultrasound waves are reflected back from an interface and travel past each other in opposite directions. This may be the case, for example, in a small container of water in the absence of absorbing materials. In medical diagnostic and therapeutic usage (generally in the range 1 to 10 MHz) the usual situation is a predominantly progressive wave field. There may be an appreciable standing wave component if, for example, there is a bone/tissue or tissue/gas interface involved (Hill, 1977). The possibility of the occurrence of standing waves is usually of less importance for pulsed ultrasonic irradiation with a small duty factor since they can only exist during the pulse overlap time.

2.1.3 Focused Fields

For diagnostic transducers used for pulse-echo imaging purposes the beam width determines the minimum lateral resolution capability that can be expected. For this reason, many diagnostic transducers are focused. The intensity distribution along the axis of such a transducer is similar to that for an unfocused one in that an axial intensity peak occurs at some distance from the transducer. This peak is a common feature of both focused and nonfocused fields and its existence is an important factor in the measurement of ultrasound fields and in the interpretation of some of the biological data and resulting recommendations that are discussed later. For a focused transducer the point of the last axial maximum is generally found as the smallest beam cross section (i.e., focal distance) is approached.

If F_L is the focal length of the converging ultrasound field and r is the radius of the transducer's lens, then an approximation for the radius of the focal area, R, is given by (Alonso & Finn, 1967).

$$R = F_L \sin\theta = 0.61(\lambda F/r) \qquad \text{Eq. 2.4}$$

2.1.4 Temporal Versus Spatial Parameters

The intensity of the ultrasonic field produced by the transducer also varies with time if the ultrasound is pulsed. Intensity averaging can also be carried out in the time domain and it is necessary to distinguish between time (or "temporal") average (TA) and temporal peak (TP) intensities (Kossoff, 1978; Stewart, 1979). The temporal characteristics of pulsed fields such as pulse duration and pulse repetition frequency in relation to biological effects have been reported by various investigations (Barnett 1979; Sarvazyan et al., 1980, 1981).

The ratio of the temporal peak to temporal average intensity will generally be the inverse of the "duty factor" and is typically on the order of 1000 for B-scan pulse-echo diagnostic equipment. Real time units have a higher duty factor. For pulsed therapeutic equipment the ratio of the temporal peak to temporal average intensity is typically about 5. The distinction between temporal peak and temporal average intensity is an important factor in the interpretation of some of the biological data, since some biological effects may be related to the peak pressure amplitude. This is particularly important when attempting to compare effects from diagnostic pulsed systems with low temporal average but high temporal peak values with those from continuous wave exposure systems. The importance of the temporal intensity peak has been demonstrated by Child et al. (1981) in the effects of ultrasound on Drosophila. Sikov & Hildebrand (1977) reported the incidence of some heart defects of rats exposed in utero have a better correlation with peak intensity than with average intensity.

2.2 SOUND VELOCITY

The speed at which ultrasonic vibrations are transmitted through a medium is inversely proportional to the square root of the product of the density and the adiabatic compressibility of the material. This speed of sound (c), along with the frequency (f) of the ultrasound, determines the wavelength (λ), of the waves that are propagated from the relationship

$$\lambda = c/f \qquad \text{Eq. 2.5}$$

For example, the propagation velocity of ultrasound in most human soft tissues is approximately 1570 ms^{-1} (see Table 2.1, Ragozzino, 1981), so that frequencies of 1 MHz and 3 MHz correspond to wavelengths of about 1.5 mm and 0.5 mm, respectively. Thus, ultrasonic diagnostic imaging procedures carried out in this frequency range have the potential for providing image resolution of the order of 1 mm. Knowledge of the speed at which ultrasound is transmitted through a medium is used in the conversion of echo-return time into depth of tissue being imaged in diagnostic applications. Values of sound velocity for some media of interest are given in Table 2.1. The published data are not always consistent, though the potential for accuracy in velocity measurements is greater than that for attenuation measurements due to the presence of a measurement standard (water) and the inherent details of the measurement procedure.

Ultrasound may propagate in different modes: In solids two important modes of propagation are as compressional (longitudinal) waves and in the form of shear (transverse) waves, the propagation velocities for the two modes being different. Ultrasonic energy is propagated in liquids and tissue primarily by compressional (or longitudinal) waves where the particles vibrate back and forth in the direction of propagation of the sound field. This results in the alternate compression and rarefaction of the particles in the medium. Transverse (or shear) waves in solids can be produced where the particle movement occurs at 90° to the direction of propagation. For example, at bone tissue interfaces in addition to strong reflections at oblique incidence, there is also some conversion from one wave mode to another. The transverse wave velocity is lower than that of the longitudinal wave in bone, so it is transmitted at a less oblique angle than the longitudinal wave. Table 2.1 illustrates that the values of the velocity of longitudinal sound waves in solids are the highest, those in liquids and soft tissue lower, and those in gases still lower.

Table 2.1 Approximate values of ultrasonic properties of various media. The ultrasonic frequency, f is in units of MHz

Medium	Sound velocity* c m/s	Characteristic acoustic impedance*** (Z)10 $10^3 kg/(sm^2)$	Amplitude attenuation coefficient (α) (cm^{-1}) (see note)****	Amplitude absorption coefficient(a) cm^{-1}	Reference
Dry air (20 °C)	343.6	0.45	.18	--	Evans & Bass, 1972
Water (37 °C)	1524	1524	$.00016f^2$	$.00016f^2$	Pinkerton 1949 Swamy et al. 1972
Amniotic fluid	1530	1510	$.00081f^{1.4}$	$.00081f^{1.4}$	Zana & Lang 1974 Bamber 1981
Brain	1525	1571	$.07f^{1.14}$	$.024f^{1.18}$	Goss et al. 1979
Fat	1485	1366	**$.07f^{1.1}$		Goss et al. 1978,1980
Liver	1570	1570	$.08f^{1.13}$	$.026f^{1.17}$	Goss et al. 1979
Heart			$.13f^{1.07}$	$.028f^{1.04}$	Goss et al. 1979
Kidney			$.10f^{1.09}$	$.028f^{1.02}$	Goss et al. 1979
Muscle	1590	1685	**$.11f^{1.02}$	$.06f$	Goss et al. 1978,1980
Tendon	1750	2100	$.44f^{0.763}$	$.14f^{1.17}$	Goss et al. 1979
Skull bone	3360	7392			Goss et al. 1978
Uterus	1625	1706	$.05f$		Okumura et al. 1973

Note: These values are for animal tissue and are for illustrative purposes only; published data are not always consistent. Actual measured values may show quite strong variability with factors such as tissue preparation temperature method of measurement and intensity.

*Velocity of longitudinal wave.

**Estimated from published data.

***The density (ρ) used for the determination of ρc was taken from ICRP publication 23.

****There is a large amount of uncertainty associated with some of the values for the attenuation and absorption coefficients. Some investigators in the field suspect that much of the difference between the "attenuation" and "absorption" coefficients may result from artifacts in measurement and that the differences are not entirely due to scattering (Carstensen et al., 1981). For example, phase dependent techniques will give larger values of attenuation than phase independent techniques.

2.3 DOPPLER EFFECT

The frequency of ultrasound measured by a receiver is dependent on the relative velocity between the receiver and the source of acoustic radiation. For relative velocity V_r, small compared to the speed of ultrasound in the propagating medium (c), the apparent measured frequency f_r is given by

$$f_r = (1 - V_r/c)f \qquad \text{Eq. 2.6}$$

where f is the frequency of ultrasound radiated by the source and V_r is such that it is positive when source and receiver are moving away from each other. This is exactly the same phenomena that occurs when a train whistle appears higher pitched as the train moves toward a listener, and lower pitched as it moves away.

If both the source of ultrasound and the receiver are stationary, but the acoustic wave is reflecting from a moving interface, a similar situation occurs. In this case, however,

$$f_r = (1-2V_i/c)f \qquad \text{Eq. 2.7}$$

where V_i is the velocity of the interface away from the source.

2.4 REFLECTION AND TRANSMISSION AT INTERFACES

The ratio of the acoustic pressure to the particle velocity in a medium is defined as the specific acoustic impedance for that medium. For plane waves in free field conditions it can be shown that this quantity is equal to the product of the density of the medium (ρ) and the velocity of sound (c) in the medium, which is known as the characteristic acoustic impedance for that medium (Z).

$$Z = \rho c \qquad \text{Eq. 2.8}$$

The extent to which ultrasonic energy is transmitted or reflected at an interface separating two continuous isotropic media is determined by the characteristic impedance.

When an ultrasonic plane wave meets a boundary between two different media, it may be partially reflected. The ratio of the characteristic impedances of the two media determines the magnitude of the reflection coefficient at the interface. The reflected wave is returned in the negative direction through the incident medium at the same velocity with which it approached the boundary. The transmitted wave continues to move in a positive direction, but at a velocity corresponding to the propagation velocity in the new medium. Just as in optics, Snell's law for reflection applies, and the angles of incidence and reflection are equal when the wavelength of the ultrasound is small compared to the dimensions and roughness of the reflector as illustrated in Figure 2.2. In Figure 2.2 the subscripts i, r and t refer to the incident, reflected and transmitted waves respectively and 1 and 2 to the first and second media that the ultrasound encounters. The relationship between θ_i and θ_t as given by Snell's law is:

$$c_2 \sin\theta_i = c_1 \sin\theta_t \qquad \text{Eq. 2.9}$$

The equations relating the ratios of the transmitted intensity (I_t) to the incident intensity (I_i) and the reflected intensity (I_r) to the incident intensity (I_i), respectively, are given in the following expressions (Kinsler & Frey, 1962; Wells, 1977).

$$I_t/I_i = (4Z_2Z_1 \cos^2\theta_i)/(Z_2 \cos\theta_i + Z_1 \cos\theta_t)^2 \qquad \text{Eq. 2.10}$$

$$I_r/I_i = ((Z_2 \cos\theta_i - Z_1 \cos\theta_t)/(Z_2 \cos\theta_i + Z_1 \cos\theta_t))^2 \qquad \text{Eq. 2.11}$$

where Z_1 and Z_2 are the characteristic acoustic impedances of the media on either side of the interface, θ_i is the angle between the normal to the reflecting surface and the direction of the incident ultrasound, and θ_t is the angle between the normal to the reflecting surface and the direction of the transmitted ultrasound.

For normal incidence, the angle of incidence (θ_i) and the angle of transmission (θ_t) both equal zero and the previous equations become:

$$I_t/I_i = 4Z_2Z_1/(Z_2 + Z_1)^2 \qquad \text{Eq. 2.12}$$

$$I_r/I_i = ((Z_2 - Z_1)/(Z_2 + Z_1))^2 \qquad \text{Eq. 2.13}$$

These relations can also be expressed in terms of transmitted, reflected, and incident pressures (p_t, p_r, and p_i, respectively, since

$$I = (1/2)\ (p_o^2/\rho c) \qquad \text{Eq. 2.14}$$

Where p_i is the pressure amplitude of the transmitted reflected on incident pressure of interest, we get:

$$p_t/p_i = 2Z_2/(Z_2 + Z_i) \qquad \text{Eq. 2.15}$$

and

$$p_r/p_i = (Z_2 - Z_1)/(Z_2 + Z_i) \qquad \text{Eq. 2.16}$$

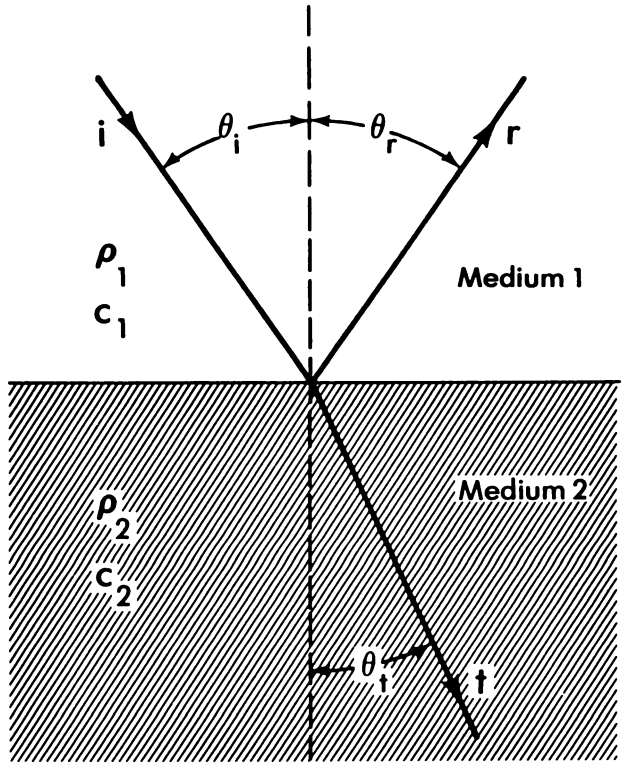

Figure 2.2. Transmission and reflection at an interface. ρ_1 and c_1 are the density and velocity of sound in Medium 1, respectively. ρ_2 and c_2 are the density and velocity of sound in Medium 2 respectively. θ_i, θ_r and θ_t are the angles of incidence, reflection, and transmission of the sound.

i,r, and t refer to the incident, reflected, and transmitted waves, respectively.

Using the value of the characteristic impedances listed in Table 2.1 and the relationships in Equations 2.12 and 2.13, it will be seen that at an interface between air and human tissue only 0.1 percent of the incident energy is transmitted, the remainder being reflected. This illustrates the importance of using a coupling medium (i.e. a liquid or gel having an impedence similar to human tissue) between the transducer and tissue for both therapeutic and diagnostic ultrasound application. Strong reflections which occur at bone/tissue and

tissue/gas interfaces serve to limit the accessibility of some human anatomical regions to ultrasonic investigation (Hill, 1977).

2.5 ATTENUATION AND ABSORPTION

The intensity of a plane progressive ultrasound field can be reduced by interaction with the transmitting medium. Two important sources of attenuation are scattering and absorption. The interface of each discontinuity within a medium serves as a reflecting surface, the size of which (in relation to the wavelength) determines its effect as a scatterer. Most of this scattered energy no longer moves only in the original direction of propagation and thus the total amount of energy transmitted is reduced. When scattering occurs, the amplitude of the scattered wave is proportional to f^n, where f is the frequency of the ultrasound and n is greater than 1. Thus greater scattering occurs at higher frequencies. The other significant source of attenuation is absorption, which occurs primarily at the macromolecular level for longitudinal waves (Carstensen, 1979; Edmonds, 1981). There are considerable differences between measured attenuation and absorption coefficients, as seen in Table 2.1. The attenuation coefficient is always larger than the absorption coefficient because absorption is only one of the means by which the ultrasound field is attenuated.

The acoustic pressure amplitude p_x of a plane progressive ultrasound wave of initial acoustic pressure amplitude p_0 and intensity I_0 at a distance x in any uniform attenuating medium, is described by the relationship:

$$px = p_0 e^{-\alpha x} \qquad \text{Eq. 2.17}$$

e is the base of natural logarithms and α is the amplitude attenuation coefficient of the medium for a given frequency. The amplitude attenuation coefficient (α) is equal to the sum of the coefficient (a) and the scattering coefficient (s) (i.e. $\alpha = a + s$) (Chivers, 1980,1981). It is a measure of the rate at which an ultrasonic wave decreases in intensity by other than geometric means as a function of distance as it propagates through a medium. Since acoustic intensity is proportional to the square of acoustic pressure, this can also be expressed in terms of intensity (I):

$$I_x = I_0 e^{-2\alpha x} \qquad \text{Eq. 2.18}$$

Where x is the distance traveled, and α is the amplitude attenuation coefficient (attenuation is also sometimes described in terms of the intensity attenuation coefficient, μ, where $\mu = 2\alpha$). Attenuation is important from several viewpoints. First, it determines the amount of acoustic energy which can reach structures of interest at various depths in a medium. For example, knowing the attenuation between the surface of a mother's abdomen and the uterine cavity determines the amount of energy reaching the fetus. Bang (1972), Kikuchi (1974), Okumura et al. (1973), Takeuchi et al. (1977), Morohashi (1977), and Etienne et al. (1976) have reported the results of such measurements (see section 2.5.2). Second, it is important because part of the attenuation is due to the absorption process in which the propagating energy is permanently converted (for example, into heat energy which causes a temperature rise in tissue) (see section 2.5.1). Third, attenuation by scattering can result in ultrasonic energy reaching structures not intended to be insonated or in production of standing waves, creating peaks and nodes in the spatial distribution of the ultrasonic energy. Fourth, most of the information presented by diagnostic ultrasound is a result of scattering.

Ultrasonic attenuation in a medium generally increases with increasing frequency in a manner that can be expressed approximately (over a limited frequency range) in the form

$$\alpha = \alpha_0 f^n \qquad \text{Eq. 2.19}$$

where α is the amplitude attenuation coefficient of the medium at a frequency f and α_o is the attenuation coefficient of the medium at the reference frequency f_o. For many human tissues and other materials of interest, n has a value between 1 and 2. Representative values of α and n are shown in Table 2.1. For therapy purposes, because of the desired depth of penetration, the useful range of frequency is about 0.5 to 3 MHz. In diagnostic use, the upper frequency limit for imaging in abdominal areas is about 10 MHz. For structures such as the eye, ultrasonic frequencies up to 30 MHz are used.

2.5.1 Temperature Elevation in Tissue by Ultrasound Absorption

Heating is an established mechanism to which ultrasonically induced bioeffects can be attributed. Several reviews of the elevation of temperature resulting from ultrasound exposure have been published (Lehmann et al., 1967; Lele, 1975; Nyborg, 1977). Effects range from beneficial therapy to localized tissue destruction and fetal abnormalities in experimental animals. Absorption is greater in some tissues than in others. This variation is exploited in therapy for differential absorption and heating of ligaments and tendons in surrounding muscular tissue (Lehmann et al., 1959; Stewart et al., 1982) .

The absorption of a traveling plane wave of ultrasound can be expressed by the following equation:

$$I_x = I_o e^{-2ax} \qquad \text{Eq. 2.20}$$

where I_x is the intensity at a distance x in the absorbing medium, I_o is the intensity at x = o, and a is the amplitude absorption coefficient.

It will be noted that equation 2.20 is similar to equation 2.18. In this case the interest is the energy deposited in the material rather than the amount of attenuation. Therefore, the absorption coefficient (a) rather than the attenuation coefficient (α) is used in equation 2.20. Absorption mechanisms in biological tissue are complicated. Table 2.1 lists the absorption coefficients for a number of biological materials. For comparative purposes with Nyborg's calculations (1977), the value of the absorption coefficient, a, will be assumed to be $0.1f^{1.1}$ nepers/cm, where f is the frequency of ultrasound in megahertz.

The decrease of intensity in the wave is accompanied by the irreversible production of heat in the tissue. The rate of heat generation per unit volume (Q) is equal to the loss of power per unit area (intensity) per unit length. Therefore:

$$Q = -dI_x/dx = 2aI_x \text{ joules/cm}^3 \text{ sec} \qquad \text{Eq. 2.21}$$

and the heat produced per unit volume per unit time is proportional to both the absorption coefficient (a) and the intensity (I_x).

The initial rate of temperature (T) rise for a given Q is given by (Nyborg, 1975):

$$dT/dt = Q/\rho h \qquad \text{Eq. 2.22}$$

where ρ is the density of the absorber and h is the heat capacity per unit mass of the absorber.

Substituting for Q we see that:

$$dT/dt = 2\,aI_x/\rho h \qquad \text{Eq. 2.23}$$

For water, and approximately for soft tissue, ρh = 4.2 joules per cubic centimeter per degree centigrade. Therefore, when tissue is the absorbing medium

$$dT/dt = 0.2f^{1.1}I_x/4.2 = 0.048\,I_x f^{1.1} \qquad \text{Eq. 2.24}$$

Based on these assumptions a 1 MHz beam with intensity of 100 mW/cm^2 will cause a rate of temperature rise of .29 °C/min. This number would increase with the frequency of the incident ultrasound. In living tissue the actual rate of temperature rise is smaller than calculated above, because of convection (blood flow) and conduction out of the insonated volume. Also, the absorption coefficient for some tissues is smaller than assumed for these calculations. Eventually, a steady state situation develops, with temperature increasing very little with time. A set of curves of temperature measurements in mice resulting from ultrasound exposure as reported by Lele (1975) is shown in Figure 2.2.

To estimate the temperature elevation upon insonation Nyborg (1977) used as a rough model a spherical body of tissue in which heat is uniformly generated by ultrasound absorption. The only assumed heat transfer mechanism is conduction (.006 W/cm °C for water, assumed nearly the same for tissue). In this case, the highest temperature (°C) rise occurs at the center of the spherical absorber and is given by $1.67R^2$ for a 100 mW/cm^2, 1 MHz ultrasonic beam, where R is the radius of the absorber in cm. Thus, for a postulated 1 cm radius absorbing sphere, the temperature rise is 1.67 °C while for a 1 mm sphere it is .0167 °C, 100 times lower. The characteristic time required for the steady state temperature distribution to develop is proportional to R^2 and is close to 700 sec for a 1 cm sphere. Figure 2.4 is a plot of a thermal threshold curve using this model.

Another model which may be used is to postulate a cylinder (corresponding to a collimated ultrasound beam) of radius R and length 2R, in which heat is uniformly generated by ultrasound absorption. In this case the temperature at the center of the cylinder is 1.3 times that of the sphere, or 2.16 °C, again for a 100 mW/cm^2, 1 MHz beam.

These models assume a uniform ultrasound beam impinging on the absorbing volume. In practice, the intensity distribution within a beam is not uniform. In the far field the distribution is approximately Gaussian. If a 1 MHz sound field is assumed (with the beam area in a plane defined as the summation of all points at which the intensity is greater than 5 percent of the peak intensity) the maximum temperature rise is 2.48 °C and 2.74 °C for the spherical and cylindrical cases, respectively. For these calculations, the average intensity over the beam area is the same for the uniform and Gaussian distributed beams.

All of the above models assume very simplified absorption patterns and no heat losses because of blood flow (convection). Insonated tissue that is well vascularized might more closely resemble a classical calorimeter, where the irradiated volume has a uniform temperature and the nonirradiated surrounding tissue remains at normal body temperature. In this case the increase of temperature would be less than calculated previously.

It should be remembered that the temperature increases calculated above were for 1 MHz, 100 mW/cm^2 average intensity ultrasound at the absorbing volume. To determine the corresponding average intensity produced by the radiating transducer one must take into account the attenuation of the tissue path the ultrasound must traverse to reach the region of interest. For 1 MHz the governing equation is:

$$I_x = I_o e^{-.2x} \qquad \text{Eq. 2.25}$$

where x is distance in cm and the attenuation coefficient is 0.1 neper/cm.

Solving for I_x = 100 mW/cm^2 and d = 5 cm we find that I_o, the average intensity produced by the transducer must be approximately 270 mW/cm^2.

In general, the energy absorption per unit volume per unit time at a distance d below the skin surface is given by

$$Q = 2aI_o e^{-2ax} \qquad \text{Eq. 2.26}$$

Q is maximized (and hence temperature elevation is maximized) when

$$a = 1/2x \qquad \text{Eq. 2.27}$$

since $a = 0.1f^{1.1}$ we have

$$f^{1.1} = 5/x \qquad \text{Eq. 2.28}$$

Thus for ultrasonic frequencies 1 MHz or greater (as is found in clinical use) the maximum temperature rise occurs at a distance of 5 cm into soft tissue. The results calculated above may then be considered a "worst case" situation with respect to frequency. Of course for a region 2 cm into tissue, 2.3 MHz radiation gives the maximum temperature increase, and, for the same average intensity output at the transducer, would be 2.5 times greater than calculated above. Since 1 °C is the approximate range of diurnal fluctuations of temperature in humans (Mellette et al., 1951; Rooney, 1980) it has been suggested that exposures to ultrasound producing less than 1 °C rise might be considered "safe" insofar as thermal effects are concerned. Using the assumptions of Gaussian distributed intensity and cylindrical symmetry the previous relations would indicate that 100 mW/cm^2 average transducer output produces approximately 1 °C maximum temperature rise 5 cm below the skin surface (1 MHz frequency). For a region of interest 2 cm below skin surface only 40 mW/cm^2 aver-age output of 2.3 MHz radiation would be required to produce this same increase of temperature.

The biological heat-induced damage depends on the maximum temperature achieved and on the length of time that temperature is maintained. According to Lele (1975) a temperature elevation of 2.4 °C (above 37°) represents a threshold for any effect to be produced, even when maintained over an extended time period. Although this should not be used in an absolute sense, it can be used for comparative purposes with thermal models. Temperature measurements at the site of the mouse fetus for cw exposure levels of 40, 80, 120, and 300 mW/cm^2 as reported by Lele (1975) are shown in Figure 2.3.

A local heating on a scale comparable to cellular dimensions (10 μm) presumably occurs as a result of local absorption mechanisms. However, because thermal diffusion distances are correspondingly small, it can be shown that the magnitude of such temperature elevations will be insignificant in practice (Nyborg, 1979; Love & Kremkau, 1980).

Calculating the temperature rise produced in tissue by sound absorption is complicated by the influences of heat conductivity and other means for heat transport. As mentioned previously, Nyborg (1977,1979) has carried out an analysis using a model in which a plane wave is assumed incident on a spherical absorber. He developed a curve giving the intensity required to produce a temperature elevation of 2.4 °C at the center of the sphere for varying lengths of exposure time. Figure 2.4 displays such a curve for a sphere of radius 1.2 cm in which the absorption coefficient is 0.1 Np/cm. This can be regarded as a model plot of a "thermal threshold" intensity-time condition for a biological system which undergoes change when the temperature rises a critical amount of 2.4 °C. Using this model and these values, at large values of exposure time, the lowest intensity for which a biological effect from a thermal mechanism is expected for a plane progressive wave is 100 mW/cm. Nyborg suggests that for this purpose the intensity should be interpreted as the spatial peak temporal average (SPTA) value (Nyborg, 1979).

Absorption in bone is considerably higher than in soft tissues. This is one reason why bone may constitute a critical organ for some forms of ultrasonic exposure, especially ultrasound therapy. As will be discussed in the section on biological effects, at levels just higher than those normally employed in physical therapy (i.e., 3 to 4 $W\text{-}cm^{-2}$), bone damage has been reported in experimental animals (Barth & Kern, 1960; Kolar et al., 1965). In addition, ultrasound exposure at a bone tissue interface can result in sudden and sometimes pronounced periosteal pain arising from a buildup of heat at the interface. This phenomenon can provide the physical therapist with an indication of potentially hazardous exposure conditions. At the bone-tissue interface some of the longitudinal oscillations (particles oscillating

in the direction of propagation) are transformed into transverse oscillations. The transverse oscillations (often called "shear waves") are more readily absorbed than longitudinal waves. This can produce local heat at the interface., which can cause periosteal pain (Lehmann, 1965; Lehmann et al., 1967).

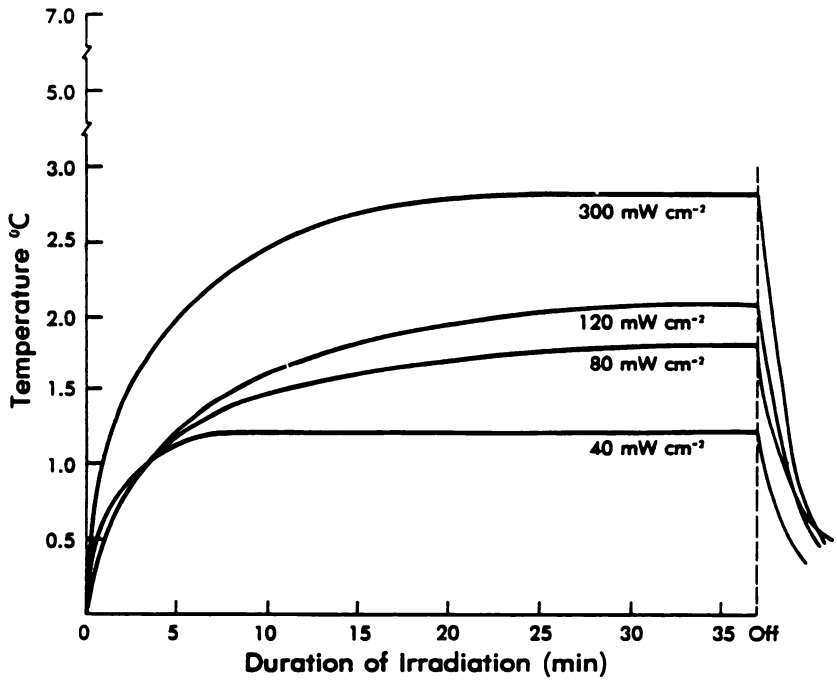

Figure 2.3. The solid lines show temperatures in mouse fetuses during continuous wave ultrasonic irradiation of mothers to varying intensity levels (from Lele, 1975). Additional points from other investigators using various conditions are plotted for comparative purposes.

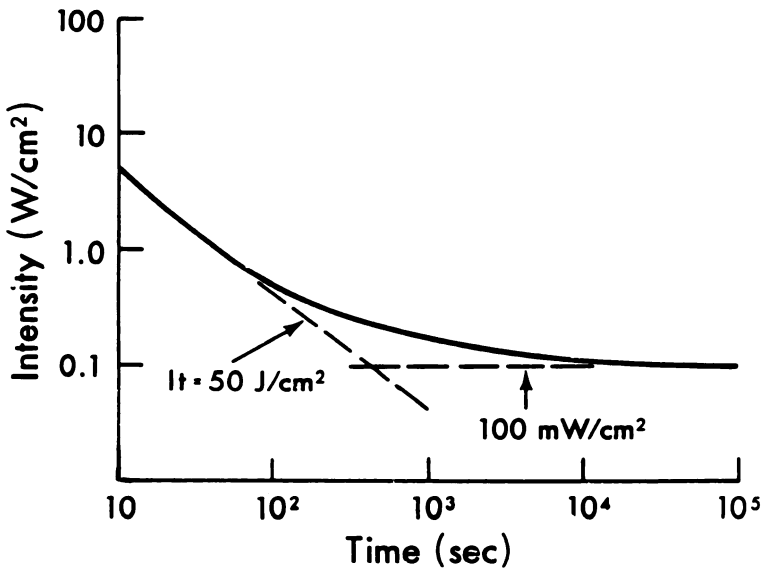

Figure 2.4. Computed "thermal threshold" curve for spherical absorber with an absorption coefficient = 0.1 Np/cm and a radius of 1.2 cm; conductivity coefficient as for water. Curve shows intensity (I) required to produce temperature increase of 2.4 °C at center of sphere after various times (t). From Nyborg (1977).

2.5.2 Attenuation Between the Abdominal Surface and the Uterine Cavity

One of the variables in comparing fetal exposure in rodents to that in humans is the degree of attenuation the ultrasound undergoes during its passage from the abdominal surface to the uterine cavity. A number of investigators have made measurements of the attenuation an ultrasound beam undergoes between the abdominal surface and the uterine cavity in women (Bang, 1972; Okumura et al., 1973; Etienne et al., 1976; Takeuchi et al., 1977; Morohashi & Iizuka, 1977). There is only one known published report on similar measurements in rodents (Bang & Northeved, 1970). Table 2.2 presents a summary of the attenuation between the abdominal surface and the uterine cavity, reported by these investigators. There is a wide variation of the reported values.

Takeuchi et al. (1977) in measurements of the attenuation between the abdominal wall and the uterine cavity in women concluded that there seems to be no relationship between the week of pregnancy and rate of attenuation. In comparison studies, the attenuation increased in all cases after abortion. The average change reported was 0.5 dB. The results of this experiment demonstrated an average rate of attenuation from 0.6 to 1.8 dB/cm at 2.25 MHz, with the total amount of attenuation averaging 5.3 dB, ranging from 2 to 7.5 dB.

The mean value of the amount of attenuation in the 2 to 2.25 MHz frequency range measured between the abdominal surface and the uterine cavity in women for the 41 measurements reported in the four papers summarized in Table 2.2 was 8.0 dB.

Table 2.2. Reported attenuation between the abdominal surface and the uterine cavity

No. of subjects	Attenuation (dB)	Distance (cm)	Average rate of attenuation dB/cm	Frequency (MHz)	Species	Reference
10	1.6 (mean)			2.25	Mouse	Bang & Northeved 1970
8	2-4	2-4.5	0.5-1	2.25	Human	Bang 1972
6	6-14	5-11	0.9-1.56	2.25	Human	Etienne et al. 1976
13	2-7.5	3-5.8	0.6-1.8	2.25	Human	Takeuchi et al. 1977
10	12 (mean)	6	.5-7.2	2	Human	Morohashi & Iizuka 1977
8	33	6	1.7-10.7	5	Human	Morohashi & Iizuka 1977

Using this value of 8.0 dB an exposure of 10 mW/cm^2 at the abdominal wall is reduced to approximately 1.6 mW/cm^2 at the fetus. The corresponding value for the attenuation between the abdominal surface and the uterine cavity in a mouse as reported by Bang & Northeved (1970) is 1.6 dB.

The intensity of ultrasound reaching the uterine cavity can also be estimated by calculations using the attenuation coefficients for various tissues (see Table 2.3) reported in the literature.

The attenuation coefficients (α) reported in the literature for human fat and muscle have been tabulated by Goss et al. (1978, 1980). There is a wide variation in the reported attenuation coefficients which may be caused by variations in freshness, temperature,

specimen preparation, measurement technique, and other factors which are all included in the data summarized by Goss et al. (1979, 1980). For example, O'Donnell et al. (1977) measured the ultrasonic attenuation coefficient of myocardium over the frequency range 2-10 MHz measured in vitro as a function of time. He reported that the attenuation coefficient was approximately independent of time for 4 hours following excision at 19.5 °C but increased by about 20 percent over a 4-hour interval at 35 °C. O'Donnell et al. (1977) also reported that the attenuation coefficient decreases approximately linearly with increasing temperature over the range 20.5° to 37 °C with the value at 37° being about 20 percent lower than at 20.5 °C. The different measuring techniques used may be one of the more important factors causing large variations in the measured values. Goss et al. (1979) reported that a difference of a factor of 3 can be obtained in the measured attenuation coefficient depending upon the measurement method used. Goss found that radiation pressure techniques yielded the lowest attenuation coefficient, possibly because of the phase insensitive characteristic of this technique. Miller et al. (1979) have indicated that phase cancellation effects associated with some attenuation measurement techniques represent the dominant source of error, resulting in values that can be too high.

Table 2.3 Ultrasonic properties of various media

Medium	Sound velocity (c) ms^{-1}	Characteristic acoustic impedance* (ρc) (z) kg $s^{-1}m^{-2}$ x 10^{-3}	Amplitude attenuation coefficient (α) vs. frequency f (MHz) cm^{-1} (see note)	Reference
Water	1490	1490	$0.00016f^2$	Pinkerton 1949 Swamy et al. 1972
Fat	(1476)	1358	0.07f	Goss et al. 1978, 1980
Muscle (gastronemius)	1566	1644	$0.11f^{1.04}$	Goss et al. 1978, 1980
Uterus	1625	1706		Goss et al. 1978
Uterus			(0.05f mean)	Okumura et al. 1973

Note: Accurate measurement of ultrasonic attenuation in human tissues is difficult and published data are not always consistent. These values are considered to be estimates only for calculation purposes.

*The density (ρ) used for the determination of ρc was taken from ICRP Publication 23 (1975).

In addition to attenuation coefficients one needs to know the anatomical construction and the thickness of the anatomical structures in order to calculate the fraction transmitted through each interface as well as the total attenuation. Using ultrasonograms it can be seen that in the transmission from the abdominal surface to the uterine cavity the ultrasound is usually transmitted through a fat layer and a muscle layer, the bladder and into the uterus. In some ultrasonograms, the bladder is not between the muscle layer and the uterus.

Using sonograms taken from the Atlas of Gray Scale Ultrasonography (pg. 280) by K. Taylor (1978), estimates of the total distance from the abdominal surface to the uterine cavity were made. The smallest total distance (without bladder) for the sonograms examined was about 2 cm. This is comparable to the smallest distance measured between the abdominal surface and the uterine cavity as reported in Table 2.2. Values of thickness

used for calculation purposes are as follows: Fat - 1.5 cm, Muscle - .7 cm, Bladder - 6 cm, and Uterus - 1 cm.

These values were chosen based on measurements from ultrasonogram cross sectional anatomy photographs, and cadaver examination. However, they should be considered only as values chosen for illustrative purposes in the following calculations and not necessarily to represent typical values. The attenuation of the pressure amplitude in transmission through a medium is given by equation 2.18. The transmission through each interface is given by equation 2.15. Most of the measured values in Table 2.2 correspond to frequencies at or near 2.25 MHz and for comparative purposes the calculations are for 2.25 MHz. Table 2.4 lists the fractional transmission through the abdominal surface to the uterine cavity using the reported attenuation coefficients from Table 2.3.

Table 2.4. Fractional transmission

	Fraction transmitted
Transmission through 1.5 cm fat-layer	.79
Transmission through fat-muscle interface	.91
Transmission through 0.7 cm muscle layer	.84
Transmission through muscle-bladder interface	.95
Transmission through 6 cm of bladder	.99
Transmission through bladder-uterus interface	.93
Transmission through 1 cm of uterus	.80
	.42
	-7.5 db

According to these calculations in traveling approximately 9 cm from the abdominal surface to the uterine cavity the pressure amplitude is reduced to 0.42 of the value at the skin or -7.5 db. The same calculation using the same thicknesses without the bladder between the uterus and the muscle wall, as can be the case in the pregnant uterus, gives a reduction in the pressure amplitude to .44 or 7.2 db.

The mean of the reported values (in Table 2.2) of attenuation measured in vivo in women from the abdominal surface to the uterine cavity (i.e., -8 db) are not drastically different than the values estimated from calculations (i.e., -7.5 db).

2.6 RADIATION FORCE

When ultrasound propagating through a medium impinges upon an object, a steady time independent force called radiation force is produced. The phenomenon of radiation force is used to measure acoustic output or intensity as discussed in section 3. In this application, the ultrasound beam interacts with a reflector or absorbing target and the force can be measured. Radiation forces are also exerted on objects in a biological medium. Gould & Coakley (1974) reported that if a suspension is diluted such that particles are widely spaced, the radiation force on each particle is exerted as if the other particles were absent. However, if particles are closely spaced, there is an interaction between the particles. Radiation force theory for closely packed particles has been reviewed by Nyborg (1978). An example of the effect of radiation force on closed packed suspension particles is the formation of particle chains in suspension referred to as the pearl chain effect (Nyborg, 1978).

Radiation force is an important mechanism by which the ultrasound interacts with biological systems. Another effect of radiation force is the blood flow stasis phenomenon reported by Dyson et al. (1974) where red blood cells in a blood vessel are brought together in parallel bands spaced at half wavelength-intervals in a standing wave ultrasound field. Radiation force has also been used to explain the flow produced in the cytoplasmic layer surrounding a large vacuole of a plant cell when radiated with ultrasound (Gershoy &

Nyborg, 1973). Spinning of intracellular bodies has also been observed and explained by the production of radiation torque when ultrasound is propagated within a liquid (Nyborg, 1965). This results from a twisting action which may be exerted on suspended objects within the liquid itself. For an asymmetric object this radiation torque varies with the orientation of the object relative to the field so that the object may tend to assume a "favored" position in which the torque on the object is minimized. Such an effect may be important in biological systems. In his analysis, Nyborg indicated that spinning of a spherical object is expected when it is near a boundary at which a wave is reflected obliquely. Dyer (1972) reported that the incipient cross wall of moss protonema was set into steady rotation about the axis perpendicular to the protonemal axis when exposed during cell division. Martin et al. (1978) has also reported the effects of radiation torque in the leaves of elodea and root tip vicia faba when exposed to 2.1 MHz ultrasound at intensities of 43 mW/cm^2.

2.7 CAVITATION

When an acoustic wave propagates in a liquid medium, the medium is subjected to increases and decreases in local pressure during the compression and rarefaction phases of the wave. This can result in the production of either (1) transient (i.e., collapse) cavitation which occurs at relatively high pressure amplitudes or (2) stable (i.e., noncollapse cavitation which is important at relatively low pressure amplitudes.

The physical conditions under which cavitation may occur in biological media appear to be quite variable and are not known in detail, although the results of some relevant investigations have been reported (Hill, 1972; Lehmann & Herrick, 1953; O'Brien et al., 1979; Webster et al., 1978; Carstensen et al., 1979; ter-Haar & Daniels, 1981).

2.7.1 Transient Cavitation

The violent phenomenon referred to above termed transient (or collapse) cavitation is important at relatively high acoustic intensity levels. In the final stages of collapse of a cavity, all of its kinetic energy can be dissipated in an extremely small volume resulting in localized high pressures and temperatures. These cavities can be formed either from vapors of the liquid or from dissolved gases or minute bubbles in the medium itself. The first case is referred to as vaporous cavitation and requires rather high intensities. The next two are referred to as gaseous cavitation and require relatively lower intensities. Vaporous cavitation does not require several cycles of a sound wave to produce a cumulative effect such as bubble growth by rectified diffusion. Rather the transmission of a single dilational pulse can produce the effect if the tensile strength of the medium being irradiated is exceeded during the rarefaction half cycle.

Hill (1972) using his own experimental criteria determined the cavitation threshold intensity (SATA) for cw ultrasound in air-equilibrated water. The threshold to produce collapse cavitation was reported to vary from about 0.1 W/cm^2 to 4 W/cm^2 over the frequency range from 0.25 to 4 MHz. The lower the frequency the lower the intensity required to produce cavitation. For continuous wave exposure, the threshold for the production of transient cavitation is at about 1 MHz never much less than 1 $W\text{-}cm^{-2}$ (Hill et al., 1969; Nyborg, 1979). At this intensity, transient cavitation in water has not been reported to be produced by the short pulses which are characteristic of pulse-echo equipment used in medical diagnosis (Nyborg, 1979; Hill, 1977; Flynn, 1975; Flynn, 1975a). However, recent findings (for example by Carstensen et al., 1980) suggest that short pulses can cause significant cavitation activity, but it is not known whether to regard this activity as transient or not. Ciaravino et al. (1981) have recently postulated a model for pulsed enhancement of acoustic cavitation. Carstensen claims that collapse cavitation can occur for pulses as short as one cycle at SPTP intensities as low as 10 W/cm^2 (Carstensen, 1981). He points out that the SPTP intensities for some diagnostic units exceed this level and can even exceed 100 W/cm^2. For some data in the literature the dependence of cavitation destruction is reported to be a function of $(I\text{-}I_t)^2$ where I_t is the value of intensity at the

cavitation threshold (Margolis, 1976). Apfel (1981) has derived an approximate expression for the threshold pressure for transient acoustic cavitation.

Investigations based on both physical measurements and morphological findings by Hug & Pape (1954) reported cavitation in tissues at intensities which are used for therapeutic purposes. They concluded, based on threshold studies, that the development of structural damage connected with cavitation requires an average intensity of more than 1.5 W/cm^2 with the transducer in a stationary position. Lehmann & Herrick (1953) have reported similar findings. Akopyan (1980) reported the cavitation threshold in potato tissue for a traveling plane wave to be approximately 1.3 W/cm^2 for exposure to 0.9 MHz ultrasound. It is not always clear if these effects are a result of transient or stable cavitation.

2.7.2 Stable Cavitation

The propagation of ultrasound through a medium will also result in the alternate compression and expansion of bubbles or cavities already existing in the medium. This activity caused by these pulsations known as stable cavitation can be relatively gentle or violent depending on the time varying pressure produced by the ultrasonic field. This type of cavitation is important at relatively low intensity levels. Cavitation (Nyborg, 1977; Coakley & Nyborg, 1976; Flynn, 1964; Carstensen et al., 1979) involving the rapid oscillation of small bodies of gas- or vapor-filled cavities of microscopic size is associated with phenomena capable of causing functional changes in biological cells. Lele (1978) measured half-harmonic emissions in brain and liver being irradiated at 2.7 kHz to confirm the existence of stable cavitation in vivo at intensities of 100 mW cm^{-2} to 1500 W-cm^{-2}.

A necessary condition for stable cavitation activity is the presence of suitable gas- or vapor-filled bubbles. They can be grown to resonant size depending on the intensity and the frequency of the ultrasound, if smaller bodies (cavitation nuclei) are present. As a rough guide, the resonant diameter of a cavitation bubble in water irradiated at 1 MHz is 3.5 μm. Neppiras (1980) has recently reviewed theories that show a stable cavitation threshold for bubbles well below resonant size. The theory suggests that when the acoustic pressure is below this threshold, bubbles decrease in size and are no longer active. An important property of a liquid medium in determining its cavitation potential is its state of nucleation (i.e., the distribution of small gaseous cavities) (Nyborg, 1977). Little is known about the state of nucleation in animal tissue. However, decompression studies have shown that nucleation sites for bubble formation do exist (Daniels et al., 1980). It is known that bubbles can develop in human tissue in vivo that are subjected to a rapid reduction in ambient pressure. This occurs in divers returning too rapidly to the surface or fliers climbing quickly to high altitudes and is known as the "bends" (caisson disease). The production of bubbles in mammalian tissue has been observed at cw SATA intensities as low as 80 mW/cm^2 for 0.75 MHz ultrasound (ter-Haar & Daniels, 1981).

Some investigators have observed a hydrostatic pressure dependence for some of the reported biological effects suggesting the effects observed could possibly be due to cavitation (Lehmann et al., 1953; Carstensen, et al., 1979; O'Brien et al., 1979). Hug & Pape (1954) and Lehmann et al. (1953, 1954) have reported effects in mammalian tissue attributed to cavitation. Other investigations also have explored cavitation as a mechanism for biological effects of ultrasound in plant tissues (Carstensen et al., 1979). In experiments using pea roots, pulse lengths of 30 to 100 μs reduced the effect of ultrasound exposure on growth rate (Child et al., 1975) and mitotic index (Arcuri et al., 1976) by a factor of approximately one-third but did not completely eliminate it. Clarke & Hill (1970) used tissue culture cells in suspension and reported that pulse durations below 1 ms produced no evidence of the effect of ultrasonic disruption of cells.

When a gas bubble is set into oscillation by an ultrasound field, its motion is not always spherically symmetric, either because of distortion by an adjoining solid boundary, or because of surface waves at the bubble-liquid interface. This asymmetric or nonuniform oscillation of the interface causes a steady eddying motion to be generated in the immediately adjoining liquid. The motion produced is usually of small spatial extent and is

referred to as microstreaming. Microstreaming can produce high velocity gradients around pulsating bubbles at the surface of a solid boundary. Microstreaming can also occur within a biological cell by a small pulsating bubble next to a cell membrane causing it to vibrate. Macromolecules or small biological cells suspended in a liquid medium may be pulled by the eddy into a region of high velocity gradient. In this situation the biological system will be subjected to shearing action and damage may occur, such as fragmentation of macromolecules or cell membranes (Nyborg, 1978).

Experiments using plant tissue and cell suspensions may provide insight concerning possibilities which may exist when cavitation nuclei are present. Miller et al. (1979) recently reported that significant biological effects could be expected in suspensions near resonant bubbles, even at low intensity levels. They found that blood platelets tended to aggregate around pulsating gas bubbles in a membrane. Martin et al. (1978) have also demonstrated this phenomenon in plant systems with experimental conditions such that the existence of gas bubbles was assured. As discussed in the AIUM/NEMA Standard (1981), the results of these and other experiments show that because of intercellular gas, intracellular eddying occurs at intensities as low as 30 mW/cm^2 and cell destruction can result at intensities as low as 75 mW/cm^2.

Rooney (1970) found that canine red cells were lysed by a single air bubble (at its resonant size) by mechanical stresses produced by cavitation at low intensities of 20 kHz ultrasound. The intensity level was below that required to produce transient cavitaton, but could cause shearing stresses set up by microstreaming near the bubble (Nyborg, 1977).

The shearing stresses occurring near a bubble that has been set into pulsation by an ultrasonic field have been studied in detail by Nyborg (1965, 1977). From approximate theory for a bubble resting on a rigid boundary (Nyborg, 1977) the amplitude of the shearing stress S produced at the boundary is given by

$$S = \omega \eta B A^2 / R \qquad \text{Eq. 2.29}$$

where ω is the angular frequency of the bubble vibration, η the shear viscosity coefficient for the liquid, B the reciprocal of the acoustic boundary layer thickness, A the radial displacement amplitude of the bubble surface, and R the radius of the bubble (in the absence of pulsation).

Since the acoustic boundary layer thickness is given by $(2\eta/\omega\rho)^{1/2}$ where ρ is the density of the fluid (Nyborg, 1977), Equation 2.29 can be rewritten as

$$A/R = (S/\omega R)^{1/2} (2/\rho\omega\eta)^{1/4} \qquad \text{Eq. 2.30}$$

This gives the radial displacement amplitude, A, required to produce a stress, S, just outside the bubble and near the boundary on which it rests. Consider blood, for example, with a fluid density of 1.0 g/cc, and a viscosity in circulation estimated to be 0.04 poise. Suppose a resonant bubble with a radius between 1.3 and 1.4 μm is driven by a 3.0 MHz ultrasound field and suppose also that the stress required for damage is 800 $dyn\text{-}cm^{-2}$; this is intermediate between quoted stress-values for red cells and those for platelets (Nyborg, 1977; Dewitz et al., 1978,1979; Glover et al., 1974; Anderson et al., 1978; Brown et al., 1975). Setting S equal to 800 and calculating the relative amplitude A/R, one obtains a value of 0.023. At very low pressure changes where the change in bubble size with acoustic pressure is essentially linear, the pressure amplitude p in an ultrasound field required to produce this oscillation amplitude is given by (Coakley & Nyborg, 1976).

$$p = 3\gamma d h A / R \qquad \text{Eq. 2.31}$$

where γ is the ratio of the specific heat for the gas in the bubble, d is the damping constant of the bubble, and h is the hydrostatic pressure. For this example, estimating γ to be 1.4, d to be 0.14, h to be 1 atm, and using the previous calculated value of A/R (i.e., 0.023), then substituting these values into equation 2.31, the driving pressure is about

0.01 atm. In a traveling plane wave field the intensity corresponding to this pressure amplitude is approximately 0.1 $mW\text{-}cm^{-2}$. As Nyborg (1977) points out, it is startling to find possibilities for bioeffects at such low intensity levels. In these calculations theory is being greatly stretched. A condition for accuracy of the streaming theory is that R should be much greater than the boundary layer thickness B^{-1}. This is not always satisfied. Also "d" is probably greater than 0.14 but has been measured for such a small bubble. However, even a more conservative estimate leads to a "startingly low" estimate of the intensity. Some of the reasons Nyborg (1977) suggests be considered for the discrepancy between the level calculated using theory and the lowest levels at which effects have been reported are:

"(1) The theory presented here involves a number of approximations and extrapolations. Neither the analysis for bubble dynamics nor that for acoustic streaming have been tested at megahertz frequencies.

"(2) Perhaps bubbles resonant to megahertz frequencies and situated near a suitable boundary do not exist in experimental situations at low levels.

"(3) The scale of distance for the expected microstreaming at 1 MHz is small; because of this a given magnitude for the stress may be less effective than at low frequencies.

"(4) Perhaps changes do occur but have not been detected."

2.8 FINITE AMPLITUDE BEHAVIOR

Two important assumptions were made in performing the calculations to determine the mechanical properties of displacement, velocity, acceleration, and pressure discussed in section 2. They were: first, that the medium in which the ultrasonic energy propagates responds linearly to stress; and second, that the wavefronts are plane. The first assumption means that the material obeys the linear Hooke's law relationship. With the intensity levels employed therapeutically, and especially the peak intensity levels employed diagnostically, appreciable departure from linear response is possible. Such nonlinear response results in the production of higher harmonics and this is known as finite amplitude distortion.

In linear acoustics, two other familiar assumptions are made: (1) the transmitted frequencies are the only frequencies propagated and (2) when the initial pressure amplitude is increased, the amplitude at remote points in the field increases proportionally. These linear assumptions are no longer valid when considering fields which are not infinitesimally small. They fail because the group velocity at a point on an acoustic wavefront is not constant (as required by linear theory) but depends on the pressure amplitude at that point. For a more detailed explanation, refer to Chapters 7 and 8 of Beyer & Letcher (1969).

A convenient way to illustrate finite amplitude distortion is to consider a wavefront that is initially sinusoidal. Finite amplitude distortion occurs because propagation velocity varies with pressure amplitude (i.e., is higher for the high amplitude portions of the pressure cycle than it is for the low pressure portions). Thus, the positive (compressed) portion of the wave advances faster than the negative (rarefied) portion of the wave eventually creating a discontinuity at the zero pressure (or static pressure) crossover point. The initial sinusoidal waveform is distorted into a shock wave that resembles a sawtooth. Since the temporal characteristics of the waveform are distorted so are its frequency components. For continuous wavefronts, analysis shows components of the frequency distortion to be integrally related to the initial frequency (Beyer, 1974).

The distortion of sound waves as a result of their finite amplitude is well known and has been under considerable study over the past three decades particularly by researchers in the field of underwater acoustics (Mason, 1965; Beyer & Letcher, 1969; Rudenko & Soluyan, 1977; Carstensen et al., 1980; Blackstock, 1966; Beyer, 1974; Shooter et al., 1974). The

acoustic intensities employed in sonar (water has relatively low attenuation coefficients) are such that finite amplitude distortion is significant.

A recent paper (Muir, 1980) shows that the frequencies and intensities used in diagnostic ultrasonics can create significant distortion of sound waves in water. However, whether or not significant distortion can occur in biological tissue where there is more attenuation has not been shown.

A significant consequence of shock formation is nonlinearly induced attenuation. As portions of the wave creep toward the shock front, they encounter a discontinuity in pressure that represents a dissipative barrier. This barrier is really a consequence of the harmonics that are produced. When the shock forms, new frequency components are generated that decay at their own individual rate, determined by the attenuation coefficient of the medium at that frequency. This loss at higher frequencies greatly accelerates the normal attenuation associated with the fundamental frequency. If the attenuation coefficient of the medium is low, more energy will be produced at higher frequencies; if the attenuation coefficient is larger, less energy will be produced at higher frequencies. If the attenuation coefficient becomes too large, then the shock will never form since linear attenuation will be more significant than nonlinear distortion (Trivett & Van Buren, 1981).

Because of nonlinearly induced attenuation, shock waves are inherently self-destructive, thus placing a restriction on propagation of intense beams over appreciable distances. As a result, there is a limit to the maximum intensity that can be produced by a given frequency of ultrasound at long ranges. This phenomenon is called saturation. It has been shown (Muir & Carstensen, 1980) that 50 W/cm^2 of 4 MHz sound radiating into water will produce 0.6 W/cm^2 at a range of 50 cm. Further increases of the initial intensity will not increase the intensity at 50 cm.

Finite amplitude effects can also potentially affect the resolution of focused systems used to visualize soft tissue (Muir & Carstensen, 1980). For strongly nonlinear systems, the fundamental and its harmonics undergo a broadening of their beam patterns and an increase of relative intensity within their minor lobes. In addition, nonlinearly induced absorption can significantly reduce the intensity gain at the focus. However, for weakly nonlinear systems the role of diffraction may increase the gain at the focus and sharpen the beam pattern (Muir, 1980).

With respect to nonlinearly induced absorption in water, as an example, the losses at the intensity of 4 W/cm^2 (1.5 MHz) can increase by more than an order of magnitude over the small amplitude losses (Krassilnikov et al., 1957) Biological media are more highly attenuating than water and this tends to inhibit nonlinear distortion. However, recent measurements have shown certain biological media to be more nonlinear than water (Law et al., 1981). The balance between attenuation and nonlinearly induced distortion in tissue is a subject of current research.

2.9 EXPOSURE METHODS AND CONDITIONS

There are several different types of exposure apparatus that may be used for biological effect investigations and medical applications. They can be organized in three categories: anechoic, echoic, and miscellaneous. Under the anechoic methods, reflections that cause interference patterns are minimized by lining the exposure tank with absorbing materials. Such exposures can be made close to the transducer (i.e., in the near field) or further from the source in the far field. If the specimen is placed close to the transducer, the exposure field is complex and may exhibit several pressure maxima and minima, near the vicinity of the specimen. In contrast, far field exposures have relatively uniform pressure distributions and can be accurately characterized, at least in the absence of the specimen.

In bioeffects investigation, anechoic exposure conditions are designed to minimize or eliminate reflections so that exposures can be made under well-defined free-field

conditions. Such exposures are generally performed in an anechoic tank lined with absorbing material and are made at a sufficient distance to be in the far field of the beam. In this way, there is a relatively uniform pressure distribution that can be accurately characterized prior to placing the specimen in the field. A number of these systems have been reported in the literature, some using focused, and others nonfocused fields (Lele, 1962; Fry & Dunn, 1962; O'Brien et al., 1974).

A typical example of this type of apparatus has been described in detail by O'Brien et al. (1974). Briefly, this system consists of a tank that is partitioned into two sections: one filled with degassed isotonic saline solution and one filled with castor oil. An acoustically transparent window separates the two sections. Ultrasound is generated at one end of a water-filled tank by a piezoelectric ceramic transducer and absorbed at the other end by the castor oil absorbtion chamber. In an ideal anechoic tank, all the ultrasound produced by the source is absorbed at the walls of the tank, thus eliminating standing waves. The acoustic field produced this way is sometimes referred to as a free field exposure condition since there are no reflected waves to cause interference.

When using an anechoic tank for biological investigations, both near field and far field exposures have routinely been performed. For the far field exposures, the free field beam characteristics can be accurately measured. In principle, the exposure field can be made as uniform as desired by moving farther from the source, although this approach has the undesirable side effect of decreasing the on-axis intensity received by the sample for a constant input level. For near field exposures, the acoustic field is certainly nonuniform, often exhibiting many pressure maxima and minima. In this case, the free field beam characteristics may not be completely determined, because they are affected by many variables (for example, transducer clamping) and because they are difficult to measure directly. Although the dosimetry is generally more difficult for near field exposures, humans are often subjected to this type of field during typical diagnostic or therapeutic treatments.

When comparing biological effects, trying to identify mechanisms of interaction or attempting to assess risk, the temporal characteristics of the ultrasound field as well as the spatial characteristics may be of importance (i.e., whether continuous wave or pulsed exposure conditions are involved). The comparison of time average intensities for these two different exposure conditions may be misleading if the effects under investigation are related to the temporal peak intensity rather than the temporal average intensity.

Echoic (i.e., nonanechoic) exposure methods are those in which reflections that can cause interference patterns or standing waves are not minimized. This category also includes direct contact exposures where the transducer is coupled directly to the subject using a coupling gel. Most humans are exposed this way during medical procedures.

Exposures involving human investigation or treatment and those in biological effects studies employ either continuous wave or pulse exposure conditions. In practical applications they may result in reflections causing artifacts and/or multiple exposures. These are called echoic exposure conditions. In some situations using continuous waves, standing wave patterns may result. Such conditions are sometimes intentionally created within the exposure apparatus during biological effects investigations (Miller, 1976). This type of system has been used to study the effects of blood flow within a chick embryo (Dyson et al., 1974). The large pressure amplitudes in a standing wave field can cause blood stasis. An example of a method that may unintentionally produce standing waves is the direct contact method in which echoes come from the finite extent of the sample or from strong reflectors such as bone (Shoji et al., 1975; Edmonds, 1980). These conditions which are similar to those using diagnostic and therapeutic application can produce exposures that are effectively higher than those expected in anechoic conditions.

An example of another medical exposure procedure has been reported by Dyson et al. (1976) in a study to stimulate healing of varicose ulcers. For this study, coupling cream is applied to the intact skin around the edge of each ulcer. This cream melts at body

temperature and partially penetrates the skin, while leaving a thin surface film through which ultrasound easily can pass. A typical treatment lasts 5 minutes, during which the applicator is moved around the edge of the ulcer in a spiral fashion.

Christman (1981) has developed another type of exposure apparatus for biological effect investigations that uses a diffuse field. This type of exposure field can be considered to be a superposition of many standing wave fields such that the acoustic energy density is distributed uniformly throughout the coupling media. A diffuse field is created by designing a nearly lossless exposure tank and by frequency modulating the transducer excitation signal using a pseudorandom white noise generator. This breaks up pressure peaks and nulls normally produced by a single frequency excitation. The advantage of using a diffuse exposure field is that it allows the measurement of average dose received by a biological specimen.

Two different types of exposure methods have already been discussed: one in which reflections are minimized or eliminated, the other producing standing wave fields or diffuse fields. Other exposure systems also exist that do not fit into either of these two categories. These include oscillating bubbles, gas-filled pores, oscillating wires, and Mason horns. Gas bubbles or gas-filled pores serve as a secondary source for ultrasound. An example of this type of exposure apparatus has been reported by Miller et al. (1979). A similar exposure apparatus has been reported by Rooney (1970). In the latter system which was limited to a maximum driving frequency of 20 kHz a gas bubble was formed at the end of a stainless steel tube, whose diameter is 260 μm, by injecting gas through the tube. An external acoustic field was used to force the bubble to oscillate.

Another exposure apparatus also operating at 20 kHz used a transversely oscillating wire bonded to a piezoelectrically or magnetostrictively driven rod (Williams et al., 1970). The transversely oscillating wire is primarily used to study biological effects thought to be associated with microstreaming.

The final exposure apparatus that will be mentioned is used to study disturbances caused by a localized divergent sound wave applied to the wall of a single cell. This type of apparatus has been used to study the effects of mechanical disturbances that can distort intracellular structures and rotate the cytoplasm. An example of this type of apparatus has been discussed by Gershoy & Nyborg (1973).

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3. MEASUREMENT OF ULTRASOUND FIELDS

As discussed previously, the acoustic pressure within an ultrasound field may vary considerably spatially and temporally. The spatial distribution of the field can be quite complicated depending on such factors as focusing, transducer radius, ultrasound wavelength, distance from the source, and even on how the transducer's element is mounted or clamped (Zemanek, 1971). It may also be expected that any biological effect and potential risk produced by ultrasound will depend quantitatively on the temporal and spatial characteristics of the ultrasonic field. It is therefore necessary to consider the methods available for making the physical measurements that will allow determination of the interrelation between equipment output levels used in human exposure and the results of biological experimentation.

In practice, these measurements are divided mainly into two parts: measurements on liquidborne and airborne ultrasound; however, this report will only address liquidborne ultrasound which is used in all current medical applications. Several extensive reviews of techniques for measuring liquidborne ultrasound have been reported in the literature (Fry & Dunn, 1962; Reid & Sikov, 1972; Hazzard & Litz, 1977; O'Brien, 1978; Stewart, 1975; Zienuk & Chivers, 1976; Wells, 1977; Stewart, 1982). The phenomenon of solid-borne ultrasound (e.g., in bone) is sometimes of interest also, but will not be dealt with here.

3.1 MEASUREMENT OF LIQUIDBORNE ULTRASOUND FIELDS

The measurement of ultrasound fields as related to biological effects should completely characterize their spatial and temporal characteristics. This will involve measurement of at least one (and possibly more) of the four field parameters (displacement (s), particle velocity (v), particle acceleration (a), and acoustic pressure (p)), discussed in section 2 as functions of space and time. Knowledge of these parameters for a plane wave field allows calculation of the spatial and temporal behavior of power and intensity (Appendix I). In order to characterize exposure completely, both the total power and the intensities as well as other factors should be specified (Stewart et al., 1977; Kossoff, 1978; Barnett, 1979; Sarvazyan et al., 1980). Acoustic power and intensity have traditionally been used for the expression of exposure. They are the parameters specified in most equipment performance standards, for example, the AIUM/NEMA Standard (1981) and the U.S. FDA's Performance Standard for Ultrasound Therapy Products. The intensities which should be specified include spatial-average, temporal-average intensity (SATA); spatial-peak, temporal-peak intensity (SPTP); and spatial-peak, temporal-average intensity (SPTA). Table 3.1 summarizes these and other factors that are important in characterizing ultrasonic exposure for investigating biological effects or assessing the potential risk that may be involved in such exposures.

Most reported biological effects experiments characterize the exposure conditions in terms of power density (i.e., intensity). Since ultrasonic absorption depends on the size of the object being exposed and the spatial distribution of the field, comparing the absorption between experiments performed with different irradiation apparatus may be difficult. Standardization in absorbed energy measurement is currently lacking, in part because of the lack of standard instrumentation or methodology for such measurement. On the other hand, absorbed energy has not yet been shown to be the most desirable physical quantity to be cited as an indicator of "potential bioeffect." It is thought to be important because of its close relationship to temperature elevation.

Relatively little has been done in the area of ultrasonic measurements in tissue. Methods for measuring ultrasonic properties of biological material have been reported (Chivers et al., 1978). Theoretical calculations to determine the ultrasonic field in simple models of tissues have also been attempted (Chan et al., 1974). Methods used for internal

measurements include thermocouples for the measurement of temperature rise at specific locations (Lehmann & Guy, 1972; Goss et al., 1977), miniature transducers inserted into bodies (Bang, 1972; Lewin, 1978), and the use of reflecting spheres (Etienne et al., 1976).

From a practical standpoint, there are basically two types of measuring instruments available. First, those that measure total power, and second, those that can measure "point" quantities (i,e., intensities over areas small compared to the dimensions of the sound field) and thus provide a capability to determine the intensity distribution of the ultrasonic field. In the next two sections examples of these types of measurement will be discussed.

Table 3.1 Some important exposure related factors

1. Exposure parameters	Unit
SPTP intensity*	$mWcm^{-2}$**
SPTA intensity*	$mWcm^{-2}$
SPPA intensity*	$mWcm^{-2}$
SATA intensity	$mWcm^{-2}$
Time average power	mW
Pulse duration	μsec
Pulse repetition frequency	Hz or s^{-1}
Duty factor	-
Frequency of ultrasound and 6 dB and 3 dB band width at transducer face	MHz
Focused or nonfocused	-
If focused focal length (and focal area)	cm (cm^2)
2. Exposure conditions needing specification	
Exposure time	
Temperature	
Pressure	
If exposure was fractionated	
Geometry of exposure conditions	
Far field or near field exposure conditions	
Source in a fixed position during exposure	
Path and speed of motion, if source moves	
Intensity constant or varying during exposure	
The degree and periodicity of the modulation or interruption, if intensity varies.	
Acoustic path length to organ or site of interest	cm
Relation of the peak to the average intensity over the beam cross sectional area of interest.	

*More fundamentally, the acoustic pressure, velocity, displacement, and acceleration (Appendix I for the relation between these and the intensity for a plane progressive wave field). Power and intensity have traditionally been used for expression of exposure in medical applications

**Note - 1 $W/m^2 = 0.1\ mW/cm^2$

3.1.1 Measurement of the Total Power of an Ultrasound Beam

Measurement of total power is important for several reasons: (1) Total power of an ultrasound field impinging on an extended plane target can generally be measured more accurately than point quantities; (2) it is commonly used to characterize standard reference

sources (such sources may be used in the calibration of detectors that measure "point" quantities, such as hydrophones); (3) measurement of the total power for a defined field size allows the calculation of the mean intensity commonly referred to as spatial average intensity. In the use of the spatial-average intensity, the beam area over which the average is measured must be specified. The beam area as defined in Appendix I is generally used. Total power is also the parameter upon which the calibration of some commercial equipment such as ultrasound therapy equipment is based (U.S. Department of Health, Education, & Welfare, 1978; Stewart et al., 1982).

As discussed in the above cited references, there are several methods capable of measuring total power including radiation force, calorimetry, and acousto-optic techniques, but the one which is usually favored is radiation force. The radiation force method is the one used for making compliance measurements for the U.S. Food and Drug Administration's Performance Standard for Ultrasound Therapy Products and is the method of choice suggested in the AIUM/NEMA Standard (1981) for measuring the total power output from ultrasound equipment. For these reasons, radiation force will be the total power measurement method discussed in most detail in this document.

For total power measurements, the radiation force technique is undoubtedly the most universally used method today. It is a relatively simple, convenient, accurate, frequency independent, and an absolute method for determining total power of an ultrasonic field. It is based on the fact that momentum is transported in a traveling plane wave ultrasonic field. If momentum is transferred at a constant rate to a reflecting or absorbing target, the target will respond as if acted on by a steady force, the radiation force (Livett et al., 1981).

The force produced is independent of frequency and is proportional to the total ultrasonic power in the field. The radiation force (F) in newtons (i.e., F=mg) is given by:

$$F = PD/c \qquad \text{Eq. 3.1}$$

where P is the acoustic power in watts, c is the propagation velocity of the wave in ms^{-1} (in water c = 1.5×10^3 meters per second at 30 °C), m is the mass in kilograms, g is the gravitational acceleration in meters per second, and D is a dimensionless factor which is determined by the type of interface encountered by the ultrasonic field and the direction in which the force produced by reflection or absorption is measured. In the following presentation, and in much of the literature, force is sometimes given in grams. This should be taken to mean the weight (force) of the mass in grams in a standard gravitational field (9.8 m/s^2). The net force, F=mg, in newtons can be measured, e.g., by opposing it on a balance with a force produced by a mass, (m in kg) in gravitational acceleration using this relation 1 newton = 102 grams. (The acceleration of gravity varies with latitude and can introduce a variation of the order of 0.5 percent.) This technique is used to measure the total acoustic power in an ultrasonic field by measuring the force on a target large enough to intercept the "entire" field. In the case where only part of the beam is intercepted, an intensity averaged over the detector area is measured. An example of this latter application involves the measurement of the deflection of a small target suspended in the sound field in a pendulum arrangement (Dunn et al., 1977).

The values for D in Equation 3.1 are shown in Table 3.2. The table has been modified from that of Hueter & Bolt (1955) to a more general one (Stewart, 1982). By knowing the type of interface a target presents to an ultrasonic field and by measuring the magnitude of the force, the total power in the acoustic field can be computed. A flat, totally reflecting plate is often used in radiation force devices. For this situation (Fig. 3.1) the only force produced by the reflected ultrasound is in a direction normal to the plate. This occurs because the component of momentum associated with the ultrasound wave is reversed in this direction but remains the same in the direction parallel to the reflector (i.e., the only change of momentum is in the direction normal to the reflecting surface, and the force produced by the ultrasound beam or the reflecting target is equal to the time rate of change of momentum). This force (F) is given by $F = 2(P/c)\cos\theta$ when θ is the angle between the normal to the reflecting surface and the ultrasound beam. If the direction of measurement

of force is not normal to the plate, only the force component in the direction of measurement will be determined. In this case the force measured is $F = 2(P/c)\cos\theta\cos\psi$, where ψ is the angle between the normal to the reflecting surface and the direction in which force is to be measured. Figure 3.1 illustrates the above discussion.

Table 3.2. Value of constant D for various physical situations for plane progressive sound field (Hueter & Bolt, 1955)

Physical situation	D**	D***
Perfect absorber, normal incidence *r = 1	1	1 cos ψ
Perfect reflector, normal incidence *r = 0 or ∞	2	2 cos ψ
Perfect reflector, not normal incidence *r = 0 or ∞ (See Notes (1) and (2))	2 $\cos^2\theta$	2 cos θ cos ψ
Nonreflecting interface, normal incidence *r = 1 c_1 not equal to c_2	$1 - c_1/c_2$ For $c_1 < c_2$ force in direction of propagation $c_1 > c_2$ force opposite to direction of propagation	

*r = $Z_2//Z_1$, the impedance ratio at an interface where $Z = \rho c$

**where the direction of sound is in the same direction as the direction in which the force is measured

***where the direction of sound is not in the same direction in which the force is measured

c is the velocity of sound in the medium

ρ is the density

θ is the angle between the normal to the reflecting surface and the incident ultrasound beam axis

ψ is the angle between the normal to the reflecting surface and the direction in which the force is measured

Note: (1) When the direction of the incident sound wave is the same as the direction in which the force is measured then $\psi = \theta$ and the value of D for a reflecting surface becomes 2 $\cos^2\theta$. This is the usual case in practice.

(2) When the direction in which the force is measured is the same as the direction of the normal to the reflecting surface then $\psi = 0$ and the value of D for a reflecting surface becomes 2 $\cos\theta$.

If $\theta = \psi$, i.e., the ultrasound beam and measured force direction are the same; the above formula reduces to 2 $\cos^2\theta$ P/c, which is the equation usually associated with these devices (Hueter & Bolt, 1955). Figure 3.2(a) illustrates this situation for a totally reflecting target. For propagation in water, a collimated beam of ultrasound exerts a force in the direction of propagation equivalent to 0.136 $\cos^2\theta$ mg/mW or 0.067 mg/mW for $\theta = 45°$. Also shown in Figure 3.2 is the situation for a totally absorbing target (Figure 3.2(b)). In this case the radiation force is independent of the angle between the target and the incident ultrasound.

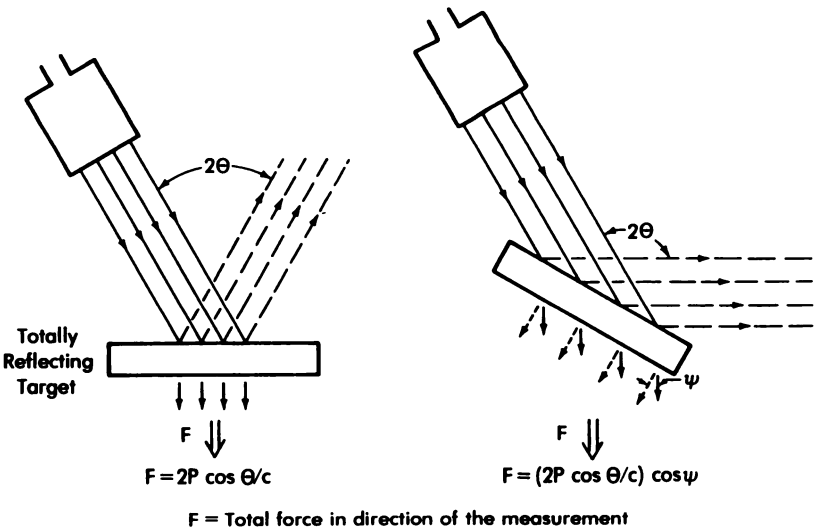

Figure 3.1 Radiation force relationships for sound incident on totally reflecting target.

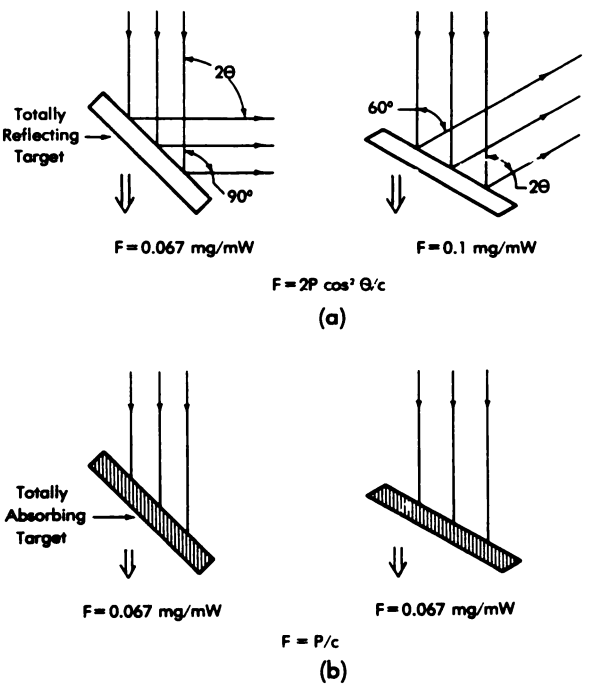

Figure 3.2 Plane wave ultrasound incident on (a) totally reflecting target and (b) totally absorbing target.

Advantages of radiation force techniques are frequency independence and that absolute calibration can be attained by weight substitution. As can be seen in Equation 3.1, calibration constants required to make an absolute calibration for a known acoustic power using weight substitution are the local acceleration of gravity and the speed of sound in the transmitting medium.

The relationship in Equation 3.1 applies for both continuous wave and pulsed ultrasonic fields, provided P is taken as a time averaged value of the beam power. Because of inertia,

the system cannot respond to the temporal variations typical of pulsed ultrasound, so the time average value of the power output is measured.

A number of practical radiation force systems for measuring the output from both therapy and diagnostic sources has been described in the literature (Rooney, 1973; Stewart, 1975; Dunbar, 1976; Robinson, 1977; Brendel et al., 1978; Carson et al., 1978; Banjavic & Carson, 1980).

3.1.2 Spatial and Temporal Measurements

To measure the spatial and temporal characteristics of ultrasound fields, a detector is needed which is small compared to the ultrasonic wavelength and which has a flat frequency response over the range of interest, high sensitivity, low noise, and wide acceptance angle. Miniature piezoelectric hydrophones, although not ideal, have generally been favored for measuring the spatial and temporal field distributions for diagnostic and therapeutic equipment. Wells (1977) describes various types of hydrophones which have been used. Devices of this type respond to the instantaneous local value of the acoustic pressure in the field. However, not all commercially available hydrophones are frequency independent and herein lies one of the primary problems in making measurements of the spectral and temporal characteristics.

The frequency response of several hydrophones has been reported in the literature (Harris et al., 1977; Lewin, 1978; Harris, et al., 1981). Most conventional probes have resonances in the frequency range of interest and distort the ultrasonic pulses being observed. The use of the piezoelectric polymer polyvinylidene fluoride as an ultrasonic hydrophone has recently been described (DeReggi et al., 1978,1981; Shotton et al., 1980; Wilson et al., 1980; Harris, 1982). Because this material has an acoustic impedance much closer to water than ceramics and because it is available in sheets having a thickness resonance of greater than 20 MHz, it promises to be useful as a broadband, acoustically transparent receiver. Hydrophones made with piezoelectric polymer are becoming commercially available. Banjavic et al. (1981) have described some of their performance characteristics.

The diameter of currently available hydrophones ranges from 0.5 to 1.0 mm. While these hydrophones have adequate resolution in the far field of a piston source in the low megahertz range, they are not satisfactory for more complex fields or at frequencies above 10 MHz. There is a need for the development of "point" detectors that give improved spatial resolution.

3.1.3 Hydrophone Calibration Techniques

3.1.3.1 Calibration by Planar Scanning a Field of Known Acoustic Power

One calibration method involves planar scanning of a beam of known ultrasonic power. A description of this technique as used by the Bureau of Radiological Health has been given by Herman & Harris (1982). The procedure involves making a series of linear, parallel hydrophone scans in a plane parallel to the source transducer face. This plane should be located at or beyond the point where the last axial pressure maximum of the radiated field occurs. The surface integral of the beam intensity distribution obtained from these scans is equal to the radiated ultrasonic power P; i.e.

$$P = \iint_S I_{TA}(\vec{r})\, dS \qquad \text{Eq. 3.2}$$

where $I_{TA}(\vec{r})$ is the temporal-average intensity at coordinate $\vec{r}$ on S, the surface of the scan plane. Assuming an incident plane wave, a hydrophone sensitivity k_f can be defined as

$$k_f = v^2(\vec{r},t)/I(\vec{r},t) \qquad \text{Eq. 3.3}$$

where $I(\vec{r},t)$ and $v(\vec{r},t)$ are the instantaneous values of the intensity and hydrophone voltage, respectively. In terms of temporal-average intensity Equation 3.3 becomes $k_f = v_{TP}(\vec{r})/2I_{TA}(\vec{r})$, where $v_{TP}(\vec{r})$ is the temporal peak hydrophone voltage. Substituting this result into Equation 3.2 and solving for k gives

$$k_f = \iint_S v_{TP}^2(\vec{r})dS/2P \qquad \text{Eq. 3.4}$$

One of the advantages of this technique is that it provides a way to relate the calibration of hydrophones to an acoustical parameter (i.e., total power) for which there are reference standards in some countries.

3.1.3.2 Reciprocity Calibration

The reciprocity technique was first applied to calibration of transducers by MacLean (1940). It has more recently been applied by other investigators to calibration of high frequency transducers such as those found in medical ultrasonic equipment (Carstensen, 1947; Reid, 1974; Brendel, 1976; Lewin, 1978; Drost & Milanowski, 1980). This method has been recommended by the IEC (International Electrotechnical Commission) (1980). In reciprocity calibration methods, one or more transducers can be used to determine a given transducer's receiving sensitivity if at least one of the transducers used is reciprocal; that is, its receiving sensitivity (voltage output/pressure input) is proportional to its transmitting response (pressure output/current input). The proportionality constant, called the reciprocity parameter, J, is a function of the measurement geometry and propagation medium characteristics. One of the advantages of this technique is that it allows measurement of the acoustic quantities from electrical and distance measurements. One of the limitations of this technique is that it is essentially confined to single frequency measurements, although the procedure can be adapted and used for the calibration of broadband pulsed medical transducers (Reid, 1974; Brendel, 1976; Drost & Milanowski, 1980). A recent comparison of the reciprocity calibration for ultrasonic hydrophones with calibration by planar scanning a field of known acoustic power has shown both methods yield consistent results (Gloersen et al., 1982). The method of choice between these methods would most likely depend on convenience, and the interest and background of the user.

3.1.3.3 Substitution Techniques

The calibration of hydrophones can also be accomplished by substitution techniques, that is, by placing the hydrophone in the field at a point of known intensity.

A radiation pressure technique for "point" intensity determinations involves the measurement of the displacement of a small ball with a diameter of between 1/2 and 1 wavelength of the ultrasound to be measured (Dunn, 1977). It is suspended in a nonviscous fluid (e.g. water), and is deflected by the traveling plane ultrasound wave to be measured.

The radiation force F in newtons (N) exerted by the deflection of a freely suspended sphere from vertical is given by the following relation:

$$F = mgd/(L^2 - d^2)^{1/2} \qquad \text{Eq. 3.5}$$

where m is the mass of the sphere plus that fraction of the suspension structure immersed, corrected for bouyancy, L is the length of the supporting string, g is the acceleration due to gravity, and d is the displacement of the sphere from its vertical position. This force F, which can readily be determined experimentally, is related to the ultrasound intensity I as follows (Dunn, 1977):

$$I = Fc/\pi r^2\gamma \qquad \text{Eq. 3.6}$$

where r is the radius of the sphere and c is the velocity of ultrasound in the fluid medium. The acoustic radiation force function, γ, can be thought of as a proportionality constant. For a given material, γ is a function of kr, where k is the wave number ($2\pi/\lambda$).

Thermal probes can also be employed in the substitution technique. In the calibration of a miniature ceramic hydrophone, Colbert et al. (1972) used a thermocouple probe devised by Fry & Fry (1954) to make the reference measurement. The thermocouple was imbedded in a sound-absorbing liquid. Placing this device in an ultrasonic beam resulted in a temperature rise at the site of the thermocouple which was related to the local intensity.

3.1.4 Reference Sources

National standards laboratories in some countries including the United States provide calibration services to measure the output of reference sources using radiation force or calorimetric techniques. They also provide reference sources with known output when a known voltage is applied. Reference sources such as these are susceptible to variations in output because of varying loading conditions, and the existence of standing waves. Therefore, care must be taken in the use of such sources. Simple direct water loading minimizes these variations, however, if they are coupled against a membrane their output may change. Such reference sources can be used to verify the accuracy of the total power measurement system or for calibrating hydrophones using the graphical integration technique. Such transducers are used by the United States National Bureau of Standards as transportable standards of ultrasonic power with estimated uncertainties of less than ± 5 percent in the frequency range from 1 to 5 MHz for power levels less than 1 watt (Zapf, 1974, 1976).

The ultrasonic beam power from these air-backed quartz transducers can be determined from electrical measurements. This method is based on a measurement of the resonant radiation conductance, G_r, of specially designed half-wave resonant, air-backed, quartz piston transducers. The radiated power is given by the following relation:

$$P_r = V^2 G_r \qquad \text{Eq. 3.7}$$

where P_r is the radiated ultrasonic power, and V the applied RMS voltage. This technique has been described by Zapf (1974), and is valid only at the frequency at which the radiation conductance is measured.

When a standard quartz transducer with a known value for G_r is used, it can be seen from Equation 3.7 that the primary source of error in obtaining a known power output will be the RF voltage measurement. One of the problems with this method is that the errors in measuring the driving voltage can be of the same or greater magnitude than the measurement of acoustic power output. Thus, some method should be used to ensure the desired accuracy of the voltage measurements. The primary standard for voltage measurement is the thermal voltage converter for accurate voltage measurement up to 30 MHz (Hermach et al., 1960). Probably the most common way in which the RF voltage is determined is with an oscilloscope (the measured peak value is converted to an RMS value) or with a true RMS voltmeter. The typical vertical gain accuracy of an oscilloscope is in the range of ± 3 percent of full scale. The typical accuracy of a true RMS voltmeter at megahertz frequencies is approximately ± 2 percent.

3.2 QUALITY ASSURANCE MEASUREMENTS

Quality assurance techniques are necessary to ensure the reliability of equipment and accuracy of calibration for both diagnostic and therapeutic ultrasound. Quality assurance testing of ultrasound equipment involves the periodic monitoring of the performance of the equipment by the operator. The monitoring should be conducted on a regular schedule, and it should incorporate state-of-the art procedures. The purpose of routine monitoring is to permit continuing evaluation of the performance of the ultrasound system.

3.2.1 Ultrasonic Therapy Quality Assurance Measurements

Quality assurance incorporates all those factors which ensure that patients receive a consistent and known amount of ultrasound exposure from a therapy applicator. It is important that the instrumentation be calibrated in order to provide the operator with the capability of delivering a prescribed amount of ultrasonic energy. The parameters that should be monitored include the output power calibration and timer accuracy. Surveys on the use and performance of ultrasonic therapy equipment have shown that this equipment may become uncalibrated without the operator's knowledge (Stewart et al., 1974a, 1974b; Repacholi & Benwell, 1979). Equipment calibration should be performed at intervals specified by the manufacturer and/or by data from previous measurements made over a period of time to determine any deterioration of calibration with time.

3.2.2 Diagnostic Ultrasound Quality Assurance Measurements

A quality assurance program is of primary importance in obtaining consistent image quality with a minimum of scanning time. Surveys of B-scan equipment performance indicate that, in some cases, diagnostic ultrasound systems in routine clinical use were performing below manufacturer's specifications (Erickson et al., 1976; Christensen & Carson, 1977). In many cases, the operator may be unaware of these deficiencies. This is unfortunate, because periodic testing of ultrasound scanning equipment can identify significant changes in system performance as they take place.

Standards of acceptable image quality should be established. The details of quality assurance measurement programs have been published (Goldstein, 1979; Lopez & Smith, 1979; Carson & Dubuque, 1979). Examples of parameters that may be monitored include the following:

1. Sensitivity
2. Axial Resolution
3. Lateral Resolution or Beam Width
4. Dead Zone or Ring-Down Distance
5. Registration or Positional Coincidence
6. Calibration of Distance Markers
7. Dynamic Range Capability
8. Scan Converter Integrity
9. Transducer Frequency Calibration
10. Film Processing Accuracy

Methods of measuring these parameters have been reported in the literature (AIUM, 1975; Carson, 1976; Nigam, 1976; Smith et al., 1977; Goldstein & Langrill, 1978; Banjavic et al., 1979). Also, the AAPM (American Association of Physicists in Medicine) (1979) has developed a draft document describing acceptance tests for pulse-echo ultrasound imaging systems.

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4. MEDICAL APPLICATIONS AND USE OF ULTRASOUND

There are two broad classifications of medical applications of ultrasound. The first is that area where there is intentional exposure and the second is the unintended incidental exposure that occurs as a result of its use in some application. Examples involving direct intentional exposure include ultrasound therapy, diagnostic applications, surgical techniques, and dental applications. An example involving unintentional exposure is exposure of a therapist's hand in a water bath to reflected ultrasound while treating a patient.

In the following subsections, descriptions are given of the applications of medical ultrasound. The coverage is not necessarily comprehensive, and, therefore, applications not discussed here might result in significant exposure to humans.

4.1 DIAGNOSTIC ULTRASOUND APPLICATIONS AND USE

This is a large and rapidly growing field and may well involve the most widespread instance of human exposure to ultrasonic radiation. At the present time, the predominant medical diagnostic application of ultrasound is in the frequency range of 1 MHz to 10 MHz, with occasional extension to 30 MHz for some eye examinations. Irradiations fall into two subcategories--continuous wave and pulsed fields. Continuous wave devices are used for Doppler shift (see section 2.3) identification of moving structures and blood flow velocity measurements, and the pulsed fields are used primarily for pulse-echo imaging which involves detecting reflections from anatomical structures. Ultrasound imaging is accomplished by scanning a pulsed ultrasound transducer over the area of interest either mechanically or electronically. When the scanning is done and the output keeps pace with changes in input, it is called real time imaging. Systems that are not real time are referred to as static scanning systems. Accessibility is impaired somewhat by bone, and to a greater extent by air spaces. The inflated lung and the gaseous bowel constitute practically impenetrable barriers. There are a number of reviews of the clinical applications of diagnostic ultrasound (Brown, 1972; Feigenbaum, 1972; Goldberg, 1977; Taylor, 1978; Lyons, 1979; Birnholz, 1980; Hohler, 1980; Nolan et al., 1981; ACOG, 1981; Nelson, 1982). Table 4.1 lists some of the applications of diagnostic ultrasound as summarized from the literature (Lyons, 1979; Stephenson & Weaver, 1981).

As this technology becomes more widely available and as technical improvements permit, the diagnostic applications of ultrasound are expected to increase in number and scope. For example, the Electronics Magazine in its annual "U.S. Marketing Forecast" (1981) indicated that by 1982 the dollar sales for diagnostic ultrasound equipment will exceed that of x-ray equipment. As shown in Table 4.1, ultrasound is used to diagnose pregnancy problems, cardiac and vascular problems, and abdominal tumors. It is also used to monitor fetal heart rate at delivery and measure blood flow in various circumstances.

Whenever new devices are developed, patterns of use can change rapidly and extensively. In the past, imaging equipment has been generally confined to hospital centers. Recent marketing of imaging and Doppler devices at relatively low cost is making it possible for obstetricians to have the equipment in their private offices, thus increasing the possibility that large numbers of fetuses will be exposed to ultrasound radiation. Exposure intensities and the amounts of energy delivered to the fetus by the Doppler, real-time and B-scan devices may be quite different.

Table 4.1 Partial list of applications of diagnostic ultrasound

Part of interest	Measurement made
1. Head	
	-echoencephalography (head scan, and brain scan) includes midline determination and ventricular size
Brain	-neonatal and newborn brain tomographic scans
	-hydrocephalis evaluation
2. Eyes and orbit	
	-ophthalmic echography (eye scan) includes tomography, ultrasonic biometry; foreign body localization, mass evaluation, retinal detachment
3. Neck	
	-arterial flow studies, plaque evaluation -carotid artery
Thyroid	-thyroid echography (thyroid scan) -mass evaluation
4. Chest	
Heart	-echocardiography (heart scan) includes -pericardial effusion -valve investigation -wall evaluation (motion, thickness) -chamber size and function -tumor detection
Pleural space	-effusion localization
Breast	-breast echography (breast scan) -mass evaluation
5. Abdomen	
Liver	-evaluate size, parenchyma and associated masses
Kidneys (renal)	" " " " "
Pancreas	" " " " "
Spleen	" " " " "
Gallbladder	-stone detection
Biliary Ducts	-evaluate size
Aorta	-aneurysmal dilation
Peritoneal Space	-ascites and abcess detection

Table 4.1. Partial list of applications of diagnostic ultrasound (cont'd)

Part of interest		Measurement made
6.	Pelvis	-amniosynthesis evaluation -evaluation of fetus
	Uterus (Pregnant)	-estimation of fetal age -diagnosis of multiple pregnancy -placental localization -amniotic cavity -fetal heart monitoring -fetal growth rate, molar pregnancy, ectopic pregnancy -congenital anomalies
	Uterus (Non-Pregnant)	-evaluate size of masses
	Ovaries	-following Graafian follicle development for ovulation timing
	Bladder	-tumor assessment
	Prostate	-tumor detection
7.	Extremeties	-vascular studies
	Arteries and Veins	-peripheral flow
8.	Ultrasonic Guidance Procedures	ultrasonic guidance for: -amniocentesis -needle biopsy -thoracentesis or cyst location -placement of ionizing radiation therapy fields

Projections can be made with regard to equipment use and patient exposure from several different standpoints: (1) estimating number of examinations by multiplying the total number of births by the fraction of pregnant women exposed and the average number of exposure per pregnancy, (2) projections based on equipment sales of various categories of equipment and estimated use, and (3) based on hospital ultrasound caseloads.

Census figures indicate that the number of births in the United States was 3.17 million in 1974 (Pearse, 1976; Monthly Vital Statistics Report, 1980). On the basis of the BRH study, (Roney & Albrecht, 1977) approximately one-third of these or about 1 million pregnant women had diagnostic ultrasound available to them in 1974. From sales growth for diagnostic ultrasonic equipment of approximately 20 percent per year as projected by Frost & Sullivan (1975) and reported sales by Electronics Magazine annual "U.S. Marketing Forecast" (1978, 1979, 1980, 1981), it is estimated that diagnostic ultrasound is now available to nearly all of the pregnant women in the United States.

It has been estimated that $214.4 million was spent to purchase ultrasonic scanners in 1980 (Electronics U.S. Markets Forecast, 1981). Assuming the average scanner costs $50,000, this would mean that approximately 4,200 ultrasonic scanning units were sold in 1980. Assuming 220 workdays per year and that on the average 5 patients are exposed per day, per unit, it can be estimated that the machines sold in 1980 (during 1 year's use) would account for approximately 4.5 million exposures. During the 4 years from 1977 to 1980, it is estimated that $50, $78.7, $169.6 and $214.4 million worth of ultrasonic scanners were sold

in the United States. Assuming an average cost of $50,000 per unit, an estimate can be made that these units can account for about 11 million exposures (Electronics 1978-1981). This does not include exposures from other types of ultrasonic equipment, such as Doppler equipment.

According to the survey conducted during the first half of 1979 by Technology Marketing Group Ltd., in cooperation with the Radiology/Nuclear Medicine Magazine (1979) the reported average monthly ultrasound caseload for the year 1978 was 82, 130, and 231 for hospitals with bed sizes from 0-199, 200-399, and 400- and over, respectively (Technology-Marketing 1979). The American Hospital Association Survey Hospital Statistics (1979) identifies 6,127 short-term general hospitals, 4,333 of these are in the 199 or less bed category, 1,160 are in the 200-399 bed category and 634 in the 400 and over bed category. Thus, on a yearly basis, an estimate can be made that there are approximately 4.2, 1.8, and 1.7 million caseloads for each of the three hospital groups, respectively, or a total of approximately 7.7 million caseloads annually in the United States. Although this estimate and the previous estimate based on equipment sales are at best very crude, they do give values of the same order of magnitude.

In a survey of the use of diagnostic ultrasound devices in Canada, which has a population about one-tenth that of the United States, Benwell (1981) reported between 340,000 and 620,000 patients were examined by diagnostic ultrasound in Canada in 1977 and that radiology, cardiology, and obstetrics were medical services in which diagnostic ultrasound was most used. In the study conducted by the Technology Marketing Group, Ltd., in cooperation with the Radiology/Nuclear Medicine, they reported that important users included radiologists, cardiologists, OB/Gyn specialists, ophthalmologists and neurologists, and nuclear medicine specialists.

Ultrasound Diagnostic Equipment Exposure Levels

While most manufacturers do not currently provide information on exposure levels with their equipment, ultrasonic intensity levels and total power output measurements from commercial diagnostic instruments have been reported by several investigators (Hill, 1969; Rooney, 1973; Stewart et al., 1977,1979; Carson et al., 1978; Kossoff, 1978; Zweifel et al., 1979; Leask, 1979; Carson, 1980; Schlensker & Gregor 1980). Table 4.2 summarizes some reported output levels from various types of diagnostic devices.

Pulse-echo imaging systems can be divided into two categories: real time ultrasonic scanners and static scanning systems. In an evaluation of real time ultrasonic scanners, the Scottish Health Service reported a wide variation in the magnitude of the output levels. The SPTA intensity varied from 0.028 to 11 mW/cm^2 and the SPTP intensity ranged from 0.4 to 130 W/cm^2 (Leask, 1979). In a summary of the reported outputs from 53 manually scanned imaging systems Stewart (1979) indicated that the SATA intensities ranged from about 0.07 to 10 mW/cm^2 and the SPTP ranged from 0.405 to 1,699 W/cm^2.

Continuous wave systems can also be divided into two categories: obstetrical Doppler systems and peripheral vascular Doppler systems. As shown in Table 4.2, the time averaged output level for continuous wave obstetrical Doppler units reported in the literature range from about 0.26 to 25 mW/cm^2. The time averaged output levels for continuous wave peripheral vascular Doppler units are high relative to obstetrical Doppler units, with recorded SATA outputs from 10 to 400 mW/cm^2. This is due, in part, to the sensitivity which is required to detect the small signals received from blood flow.

It is interesting to observe that many of the recent biologic effects data discussed in Section 5 are the result of exposures using pulsed diagnostic ultrasound devices (Sanada et al., 1977; Siegel et al., 1979; Liebeskind et al., 1979a, 1979b, 1982; Haupt et al., 1981; Moore et al., 1982). The peak intensities (SPTP) associated with these devices can go up to hundreds of watts per square centimeter, while the time average values remain much lower. It seems conceivable, therefore, that some biological effects may be related to the high

pressure amplitude associated with temporal peak intensities rather than the temporal average intensities.

Table 4.2 Range of ultrasonic output intensities found in beams produced by medical ultrasonic equipment

Type of equipment	Acoustic Power	*Spatial average, temporal average intensity on the radiating surface	Spatial peak, temporal average intensity	Spatial peak pulse average intensity	Spatial peak temporal peak intensity
Static pulse-echo scanners and mode equipment	0.1-20 mW	.01-10 mW/cm^2	0.6-125 mW/cm^2	.01-160 W/cm^2	0.4-700 W/cm^2
Automatic sector scanners (phased arrays and wobblers)	9-20 mW	0.5-15 mW/cm^2	2-200 mW/cm^2	0.3-75 W/cm^2	.4-300 W/cm^2
Sequenced linear arrays	0.1-33 mW	0.06-10 mW/cm^2	0.5-12 mW/cm^2	0.3-69 W/cm^2	.4-120 W/cm^2
Pulsed doppler, primarily for cardiac work	8-24 mW	5-32 mW/cm^2	20-290 mW/cm^2	1-14 W/cm^2	2-600 W/cm^2
Doppler instruments, primarily for obstetric applications	1-18 mW	0.26-25 mW/cm^2	0.6-75 mW/cm^2		
Continuous wave doppler primarily for peripheral vascular	6-105 mW	10-400 mW/cm^2			
Therapy continuous	1-25 W	0-4 W/cm^2	0-16 W/cm^2		
Therapy pulsed	.250-4 W	0-1 W/cm^2	0-4 W/cm^2		

Intensity data were obtained from published values in the literature (Carson, 1978; Etienne et al., 1976; Hill, 1969; Nyborg, 1979; O'Brien, 1978; Rooney, 1973; Stewart et al., 1977, 1979; AIUM/NEMA 1981).

*See Appendix II for definitions of various types of intensities

Efforts have been made in Japan to reduce the intensity from diagnostic ultrasound units. Morohashi & Iizuka (1977) reported obtaining clinically useful Gray scale images by a new ultrasonic B-mode manual scan with an intensity as low as 1/55th of a conventional low power device. They also reported obtaining clinically useful results using a new ultrasound Doppler fetal detector with an intensity of 0.4 mW/cm^2. In addition, Morohashi et al. (1980) reported clinically useful results from a real time B-mode unit with an SATP intensity of 10 mW/cm^2. An approach to design diagnostic ultrasonic imaging equipment for exposure reduction has also been reported in the United States by Melton (1979). Endo et al. (1973) have reported a technique for modification of commercial Doppler equipment to reduce the acoustic intensity required from 8 mW/cm^2 to 1 mW/cm^2.

4.2 THERAPEUTIC APPLICATIONS

There is a fundamental difference between therapy and diagnostic applications. Diagnostic applications involve trying to obtain information without intentionally causing a biological effect in the system. Therapy applications involve putting enough energy into the body to cause a desired biological effect.

Ultrasound therapy is one of the more widespread sources of ultrasonic irradiation to humans at power levels capable of causing heating and significant biological effects (Lehmann & Herrick, 1953; Dyson et al., 1968; Wehner et al., 1980; Stewart et al., 1980). In ultrasound therapy, ultrasonic fields in the frequency range of 0.8 MHz to 3 MHz are used with the objective of increasing extensibility of collagen tissues, decreasing joint stiffness, providing pain relief, improving mobility and reducing muscle spasms, and accelerating healing in ulcerated conditions (Lehmann & Guy, 1972; Dyson et al., 1976). Therapy exposure time periods are generally between 5 and 15 minutes per treatment, and the irradiation used can be either continuous wave or pulsed.

Ultrasound is used in hospitals, clinics, medical centers, nursing homes, private offices, health offices, health spas, and athletic departments. For example a 1972 study of ultrasound units in Pinellas County, Florida, identified users of ultrasound therapy equipment as including physicians, osteopaths, chiropractors, nurses and registered physical therapists (Stewart et al., 1974).

In a 1970 study of diathermy units in use in Pinellas County, Florida, the number of units observed was 116 ultrasonic, 67 short wave, and 73 microwave (Remark, 1971). The results of this survey indicated that ultrasonic units were most numerous and accounted for the largest number of treatments. Comparable results were obtained in surveys conducted in Massachusetts in 1970 and in the Washington, D.C. area in 1972 (Massachusetts, Diathermy Survey, 1972).

In a survey conducted by Frost & Sullivan (1975), it was estimated that the 4,600 hospitals that provided physical therapy services in 1974 had slightly over 11,000 units. Assuming that 50 percent of the remaining hospitals had a physical therapy unit, Frost & Sullivan (1975) estimated there are slightly over 12,000 units in use in hospitals. In a survey conducted in Ottawa, the average number of patients that were reported treated each week per device was 46.7 (Repacholi & Benwell, 1981). In a survey conducted by Remark (1971) in Pinellas County, Florida, there were a reported 89.5 treatments each week per device. Assuming five exposures a day for each unit in the United States an estimate can be made that these units account for about 15 million treatments a year.

Data from a survey conducted in 1977 by the American Chiropractic Association (ACA) provide information about the clinical use of this modality by chiropractors. The following information was provided by Dr. Edward C. Velie, Director of Statistics of the American Chiropractic Association. "Our sample of 2,848 chiropractors (roughly 17% of those in active practice) reported an average of 111 patient visits per week. About 47% of the respondents indicated that they used ultrasound therapy. Extrapolating these figures to the total population of chiropractors for an average 49 work-week year, and assuming (from previous work) that 38% of the patients can be expected to receive ultrasound treatment, there were, in 1977, an estimated 16 million ultrasound therapy treatments."

4.2.1 Ultrasound Therapy Equipment Exposure Levels

Typically, ultrasonic therapy units are equipped with an output power indicator (which is either a meter or calibrated dial), a timer, and a power output adjustment. They usually register total power (W) and intensity ($W\text{-}cm^{-2}$), which is the power divided by the effective radiating area of the transducer (Appendix II). Some ultrasound units can be operated in either continuous wave or pulse mode. For pulsed waveforms, the maximum value of the intensity averaged over each cycle of the carrier wave must be indicated (DHHS Ultrasound Therapy Standard, 1978). This concept is illustrated in Figure 4.1 (Stewart, 1982 and Stewart et al., 1982). In the pulse mode, most units operate at a pulse repetition rate from about 8 pps to 120 pps and with a pulse width up to 12 ms.

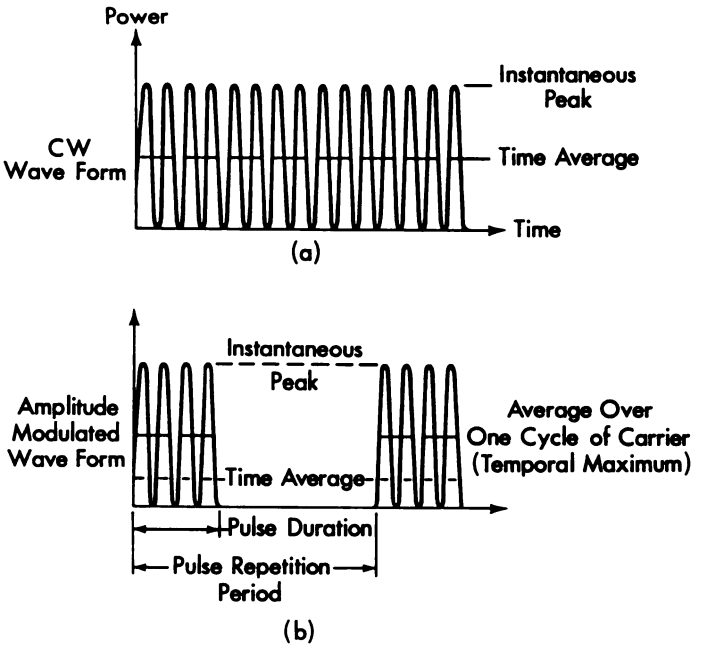

Figure 4.1 Output power vs. time for continuing wave and amplitude modulated wave forms.

In continuous wave operation, the temporal average ultrasonic power and spatial-average, temporal-average intensity can usually be adjusted up to about 20 watts and 3.0 W/cm^2, respectively. In one unit recently introduced into the commercial market, operating in the pulsed mode the peak power and temporal-peak, spatial-average intensity may be adjusted up to approximately 80 watts and 8.0 W cm^{-2}, respectively (Stewart et al., 1982). The intensities used in therapy are generally in the range of 0.1 to 3 W/cm^2. The upper limit is determined mainly by thermal considerations (Lehmann & Guy, 1972) while the lower limit seems to be a vague, controversial estimate of the minimum useful therapeutic intensity for effects that do not seem to be entirely thermal (Paroni, 1958; Paul, 1958; Paul et al., 1960; Summer & Patrick, 1964; Reid & Cummings, 1973).

Because the beam divergence is a function of applicator size for a given ultrasonic frequency, therapy transducers with beam areas of less than 5 cm^2 have been stated by some as being unacceptable (Lehmann, 1965). In addition, it may be difficult to treat an area on an individual with a small beam. On the other hand, if the radiating area of the applicator is too large it may be difficult to maintain contact with the surface of the body during treatment. The effective radiating area of therapy applicators generally ranges between 6 cm^2 and 10 cm^2.

4.2.2 Hyperthermia

A relatively new use of ultrasound is its inclusion in the medical arsenal of methods for cancer treatment. Ultrasound can produce hyperthermia in surface and deep seated tissue volumes (Lele 1967, 1980; Palzer & Heidelberg, 1973; Schwan, 1980). There is some evidence that hyperthermia under appropriate conditions can cause regression of spontaneous tumors in man (Marmor et al., 1978, 1979, 1979b, 1980). In addition, hyperthermia seems to have a synergistic effect with ionizing irradiation and chemotherapy in many cases (Palzer & Heidelberg, 1973; Witcofski & Kremkau, 1978; Marmor, 1980,1982). Deep heating of tumors by ultrasound can also be used as a noninvasive surgical modality to induce necrosis of malignant tissue (Lele, 1967).

Temperatures utilized in clinical situations or experimental studies range from 42 °C to above 50 °C. The difference in heat sensitivity between normal tissue and tumor tissue is reported to be on the order of 1 °C to 2 °C (Pettigrew, 1974). This implies that normal tissue, in the path of the ultrasound and surrounding the tumor may be adversely affected by elevated temperatures, particularly in the case of deep-seated malignancies.

Research is being conducted to investigate further the possibilities and problems of selectively heating deep-seated volumes within the body without adversely affecting intervening or surrounding tissue. Ultrasound techniques being examined include focusing, multiport exposures, arrays, and movable source configurations (Dobson, 1980; Lele, 1982).

4.3 SURGICAL AND OTHER MEDICAL USES OF ULTRASOUND

In addition to diagnostically assisting certain surgical procedures, a variety of techniques has been described in which ultrasound is used as a surgical tool. These can be divided into two groups: those in which a focused ultrasound beam is used to effect some surgical action or produce lesions in specific parts of living tissue; and those which are dependent upon the direct mechanical effects of the ultrasonic vibration of a probe (Goliamina, 1974). Few of the techniques in either group have yet achieved more than limited experimental use in the United States, however, they are in use in other parts of the world. Applications include the use of miniature beams in the treatment of Meniere's disease of the inner ear (Bullen et al., 1963; Kossoff, 1972) and surgical removal of cataracts, using an ultrasonic device that emulsifies the cataract (Kelman, 1967, 1969).

4.3.1 Treatment of Inner Ear

Meniere's disease is a disorder of the vestibular end organ, which causes spasmodic occurrences of vertigo of varying duration and severity. The effects of the disease may interfere so much with locomotion as to render the patient's life almost intolerable. In general, cases of Meniere's disease are reported to respond to ultrasound treatment (Bullen et al., 1963; Kossoff, 1972).

One treatment involves surgically exposing the lateral semicircular canal so that direct application of 3 MHz sound for approximately 20 minutes is possible at intensities that are progressively increased up to 20 $W\text{-}cm^{-2}$. Two factors involved in this type of operation for Meniere's disease are (1) that there is some risk of damaging the facial nerve indirectly by thermal conduction from its irradiated areas and (2) the operative approach to the lateral semicircular canal requires a fairly high degree of surgical skill.

4.3.2 Ultrasound Cataract Removal

Ultrasonic devices used for surgical removal of cataracts employ a small needle-like device which vibrates at about 40 kHz and is introduced into the interior chamber of the eye causing emulsification of the cataract, and the debris in situ is removed by aspiration. The results of this technique are comparable with those of conventional surgery (Kelman, 1969). However, because of the small size of the incision, the post-operative progress is more rapid, and it is reported that there are fewer operative or post-operative complications (Kelman, 1969).

4.3.3 Cutting and Welding

Surgical techniques for cutting and welding of tissue using low frequency ultrasonic probes have been used mainly in eastern Europe (Nikolaev, 1973; Goliamina, 1974; Volkov & Shepeleva, 1974). When ultrasound is used for cutting, the tendency for the patient to bleed is reportedly greatly reduced. The ultrasonic saw for cutting is reported to be 10 times faster than an ordinary saw (Goliamina, 1974). Ultrasound bone welding is used to hold the various pieces together while natural regeneration occurs. The technique is claimed to be useful in orthopedic surgery and in the treatment of complicated fractures of the skull

(Volkov & Shepeleva, 1974). For cutting, the ultrasound scalpel is driven at 20 kHz to 50 kHz to give a vibration amplitude of 50 microns to 80 microns. In the bone welding technique, a monomer of ethyl alphacyan-acrylate (cyacrine) is polymerized in less than 1 second (Volkov & Shepeleva, 1974).

4.4 OTHER MEDICAL USES

4.4.1 Ultrasonic Nebulizers

Ultrasonic nebulizers designed for medical purposes are commercially available and have been described by Boucher & Kreuter (1968). These work on the principle that when a beam of ultrasound of sufficient intensity is passed through a liquid and directed at an interface, "atomization" of the liquid may occur. Under suitable conditions, very fine, dense fogs may be produced. A water aerosol having a median particle diameter of about 4 nanometers can be generated with 1 MHz ultrasound. Miller et al. (1968) have described the maintenance of humidity using an ultrasonic nebulizer.

4.4.2 Acoustic Microscope

Acoustic microscopes are devices used to analyze various materials including tissue samples. The frequencies used by these microscopes vary from 100 MHz to 3 GHz. At the upper range of frequency the resolution of the acoustic microscope is on the order of resolution from optical microscopes. The advantage offered by acoustic microscopy is the ability to visualize through optically opaque objects, to determine the mechanical properties of the object under study, and the lack of need to use contrast stains on optically transparent objects. A review of acoustic microscopy techniques and applications can be found in an article by Kessler & Yuhas (1979).

4.5 APPLICATIONS IN DENTISTRY

Ultrasonic dental scalers were first developed in the United States in the mid to late 1950's. They are used for removal of calculus from both root and crown surfaces of teeth and also for gingival curettage (Forrest, 1967).

The use of ultrasonic dental scalers for scaling and other purposes is well documented (Johnson & Wilson, 1957; Ewen, 1959; Jarabak, 1961; Mosco & Bressman, 1964; Green & Sanderson, 1965; Forrest, 1967; Ewen, 1969; Lees, 1972).

Action of the ultrasonic dental scaler is that of a small hard object that vibrates in a sliding fashion against dental tissue surfaces at high oscillatory rates of 20 kHz to 40 kHz but at small excursions of a few tenths of microns (Frost, 1977). One of the features of ultrasonic dental scalers is adjustable water stream or mist to cool the tool tip and tissue surface. Cooling dissipates heat developed through energy loss within the transducer and through friction contact with the tooth at the tool tip.

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5. EFFECTS OF ULTRASOUND ON BIOLOGICAL SYSTEMS

The most direct evidence of any risk would be that obtained from human experience. When such evidence is unavailable, scientifically inconclusive, or ethically unobtainable, information from controlled laboratory studies must be used. Even when adverse effects are observed in human subjects, experimental evidence is necessary to establish a cause-effect relationship between the physiological agent and the biological response. This section will discuss the literature associated with the potential biological effects of ultrasound in humans, experimental biological systems, and the possible mechanisms for producing biological effects. Some of the weaknesses in these studies include: the ultrasound dosimetry was often inadequate, most of the studies did not mimic the conditions of the clinical situation, and almost none of the studies has been independently verified to date. In the case of conflicting results, studies reporting negative results will also be reviewed. In many studies, only spatial average temporal average intensities* have been reported, therefore, all exposure intensities in this review are stated as spatial and temporal averages, unless otherwise specified. In this section, the term "average intensity" means temporal average spatial average intensity. There may be, of course, major differences between the ultrasonic field parameters from a pulsed source and from a continuous wave source operating at identical SATA intensities, e.g., particle displacement, particle velocity and particle acceleration (see discussion in section 2).

5.1 PHYSICAL MECHANISMS WHICH PRODUCE BIOLOGICAL EFFECTS

Since ultrasound is a physical agent, interaction is based on purely physical principles. However, in a biological context it takes on special aspects. Although all the mechanisms by which ultrasound may produce changes in tissues are not completely understood, there are nevertheless well-understood principles which help to make predictions possible. For example, pressure, compression, stress (elastic or viscous), expansion, velocity, acceleration, tension and shearing are associated with the transmission of ultrasound through a medium. Biological changes produced may correlate with one or more of these parameters. Currently, effects on biological systems can be classified into those produced by thermal or nonthermal mechanisms. Nonthermal mechanisms include cavitation, radiation force, radiation torque, and acoustic streaming. A significant source of information on ultrasound effect mechanisms together with a discussion of reported biological effects was compiled by Nyborg (1977, 1979).

When a biological system is irradiated with ultrasound, changes may take place because of a temperature rise produced. When this happens, the change is said to have occurred by a thermal mechanism. A discussion of this mechanism has been reported in the literature (see section 2.5). Nyborg (1977,1979) has developed a thermal threshold model (see section 2.5) which estimates the lowest intensity (100 mW/cm^2) at which thermally caused biological effects may be expected. This estimation is made as a function of exposure time for a progressive plane wave field.

Because of the simplicity of the model (thermal conductivity is ignored, a plane progressive wave is assumed, the media is homogenous and has a single absorption coefficient, and a fixed volume of tissue is exposed), it must be used with caution when trying to classify biological effect experiments as hyperthermic.

While some of the existing observations of ultrasonic biological effects are consistent with Nyborg's model many are not. Stolzenberg et al. (1980) have reported a reduction in mean body weight after exposure in utero to 1 W/cm^2 for 400 seconds (see section 5.4.1.).

*When determining SATA intensity, the geometrical area of the transducer's pieozoelectric element is often used rather than the beam's cross section as defined in Appendix II.

They observed temperature rises in the uterus of more than 43 °C, suggesting a thermal effect. Stratmeyer (1979, 1981a, 1981b) has observed a weight reduction in some organs and body weight reduction of ICR mouse fetuses exposed in utero to 1 MHz cw for ultrasound on the 4th or 10th gestational day to SATA intensities of 75 mW/cm^2 for 2 minutes. These results are thought not to be due to hyperthermia because of the short exposure time and the fact that the animal's initial core temperature was depressed due to anesthesia and immersion into a 30 °C water bath. An apparent inconsistent observation is reported by Shoji (1975) of fetal abnormalities of mice exposed in utero after exposure to SATA intensities of 40 mW/cm^2 continuous wave ultrasound. However, Edmonds (1980) suggests that because the animal was not immersed in a water bath, the total output of the transducer was absorbed within the animal and the results may be caused by hyperthermia.

Nyborg's model can be improved by considering thermal conductivity, tissue structure, and the internal exposure field. To a first order approximation, internal organ exposures may be determined by modeling the animal as a series of attenuating layers of known thickness.

Some of the recently reported biological effects data obtained using pulse-echo diagnostic ultrasound equipment (Anderson & Barrett, 1979, 1981; Hara et al., 1977; Liebeskind et al., 1979a, 1979b, 1981a, 1981b; Siegel et al., 1979; Haupt et al., 1981), are at temporal average output levels below those that would be expected to result in biological effects by a thermal mechanism. This again raises the question of whether these effects may be caused by a mechanism more closely related to the temporal peak intensity (i.e. peak pressure amplitude) or other pulse parameters rather than the temporal average intensity. The importance of temporal characteristics of pulsed fields such as pulsed duration and pulsed repetition frequency in relation to biological efects have also been discussed by other investigators (Barnett, 1979; Sarvazyan et al., 1980). Lewin & Chivers (1980) have proposed a visoelastic model of the cell membrane as a potential mechanism that should be investigated in connection with pulsed exposures.

Carstensen (1982) has suggested that the temporal peak intensity rather than the time average intensity is closely correlated with effects observed when the SATA intensity from a pulsed ultrasound source is low as is the case for pulse-echo diagnostic sources. Child et al. (1981) have demonstrated the importance of the temporal peak versus temporal average characteristics of ultrasound in producing effects on Drosophila larvae. Sikov & Hildebrand (1977) reported the incidence of some heart defects of rats exposed in utero have a better correlation with peak intensity than with average intensity. At the cellular level, Maeda et al. (1977) found that exposure of intensities greater than 800 mW/cm^2 (i.e. between 0.8 and 2.6 W/cm^2) of continuous wave 2 MHz ultrasound resulted in the growth suppression of human amniotic originated cells. Using pulsed 2 MHz ultrasound Maeda & Murao (1980) observed that cell growth suppression was produced for SATA intensities greater than 38 mW/cm^2 (repetition rate 1 kHz, 3 microsec pulse duration, SATP 12.7 W/cm^2, SPTA 152 mW/cm^2, SPTP 50.8 W/cm^2).

As noted in section 2.1 the transmission of ultrasound through tissue can result in large changes in the parameters associated with particle movement (i.e., particle pressure, velocity, and acceleration). The magnitude and biological significance of changes produced may depend on both the system being irradiated and the detailed spatial and temporal characteristics of the ultrasonic field. If the changes observed after radiation with ultrasound can be duplicated by application of a stress mechanism other than ultrasound, the effect is said to have been produced by this stress mechanism (Nyborg, 1979).

As discussed in sections 2 and 3, when an ultrasonic field impinges on an object with a different density than the surrounding medium, a force or a torque will be exerted on the object. The force tends to translate the object as a whole while the torque tends to rotate the object. The red blood cell stasis in blood vessels of a chick embryo observed by Dyson & Pond (1974) is an example of a biological effect which is thought to be produced by radiation force. This effect has also been shown in mammalian vessels (ter-Haar & Daniels, 1981). An example of a biological effect of radiation torque is that observed by Dyer (1965).

When the boundary of moss protonema was insonated during cell division, this caused the incipient crosswall to be set into rotation. Nyborg (1977) and Martin et al. (1978) have also observed the effects of radiation torque.

Another type of stress, viscous stress, occurs when a nonperiodic ultrasonically induced flow of fluid known as microstreaming occurs. Microstreaming and the concomitant viscous stress occur most readily near vibrating bubbles and near solid-liquid interfaces. Sample exposure apparatuses have been designed to study the effects of acoustic streaming (Rooney, 1970; Williams & Hughes, 1970).

Cavitation (i.e., the activity of gas-filled bodies in a sound field) as discussed in section 2.7, may produce significant biological effects. There are two types of cavitation, transient and stable. For continuous wave exposure, the threshold for the production of transient cavitation in water is never much less then 1 W/cm^2 (Hill et al., 1969; Nyborg, 1979). Cavitation can produce heating, mechanical stress, and ionization. As discussed in section 2.7, some models for stable cavitation suggest that some biological effects may be possible at intensities in the mW/cm^2 range. Stable cavitation (i.e., the production of bubbles or sites) has been observed in vivo in mammalian tissue at cw SATA intensities as low as 80 mW/cm^2 (ter-Haar & Daniels, 1981).

The biological effects of ultrasound depend upon many factors, such as intensity, exposure time, the temporal and spatial characteristics of the ultrasound field, temperature, pressure, etc. Sarvazyan (1981) also points out that the formation of a biological effect depends on the dynamics of the physiochemical processes involved in the object radiated. The study and understanding of the mechanisms of biological action of ultrasound on living tissue requires knowledge of these processes as well as the more easily observed biological end points immediately following exposure. This is important because a change in a biological system resulting from ultrasound exposure may be separated in time by a long chain of physical, physiochemical, biochemical and physiological processes. Today we have only a few fragments of information concerning the processes involved in producing such effects.

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5.2 BIOMOLECULES

Many investigators feel that the primary mechanism for the absorption of ultrasound by biological tissues is at the macromolecular level. For example, it has been shown that the absorption properties of blood are determined mainly by its protein content and that the absorption coefficient is directly proportional to the protein concentration (O'Brien & Dunn, 1972; Carstensen, 1960; Kremkau & Carstensen, 1972). Furthermore, while the frequency dependence of ultrasound absorption by whole and homogenized liver tissue is very similar, the absorption coefficients of whole liver are approximately 30 percent higher than those of homogenized liver (Pauly & Schwan, 1971). These studies have led to the conclusion that approximately two-thirds of the absorption occurs at the macromolecular level, with one-third due to the tissue structure (Pauly & Schwan, 1971; Carstensen, 1960). Therefore, numerous studies have been performed using solutions of biomolecules in order to elucidate the mechanisms for the absorption of ultrasound by biological tissues.

Studies with aqueous solutions of proteins, polypeptides, and nucleic acids have not produced any clear-cut mechanism for the absorption of ultrasound in the neutral pH range. While proton transfer reactions between solute and solvent appear to be the mechanism for ultrasound absorption at pH extremes (pH less than 5 and greater than 8), these do not occur appreciably in the neutral range. Perturbation of solvent-solute interactions, intramolecu-lar hydrogen bonds, keto-enol equilibria, intramolecular proton transfer,

or viscous processes have all been suggested as the mechanism for absorption at neutral pH (O'Brien & Dunn, 1972; Zana et al., 1972; Slutsky & White, 1972; Hussey, 1972; Lang et al., 1971; Kessler & Dunn, 1969). Slutsky et al. (1980) recently demonstrated that proton exchange between phosphate ions and histidyl residues occurs around pH7 and suggested that perturba-tion of this equilibrium would be a mechanism for the absorption of ultrasound in the neutral pH range.

Intermolecular interactions also appear to be important in ultrasound absorption since it has been reported (Kremkau & Carstensen, 1972) that the specific absorption of hemoglobin solutions increases with increasing concentration of protein and that the specific absorption of dextran and polyethylene glycol solutions increases with increasing polymerization up to about 100 monomer units (O'Brien & Dunn, 1972). Furthermore, Sadykhova & El'Piner (1970) have shown that the absorption of ultrasound by a solution of F-actin (polymer form) was much greater than that by a corresponding solution of G-actin (monomer form), and Kremkau et al. (1973) have reported that fixation of erythrocytes with acrolein or glutaraldehyde, which crosslinks macromolecular hydrophobic groups, resulted in as much as a fivefold increase in the specific absorption of ultrasound compared with unfixed samples. Goss & Dunn (1980) have suggested that the ultrasonic absorption characteristics of biopolymers in solution is affected by their molecular structure as well as by their concentration. For example, dilute solutions of collagen, which is a triple helix, and DNA, which is a double helix, exhibit a greater degree of ultrasonic absorption at 6 MHz than do more concentrated solutions of less structured molecules such as hemoglobin and albumin. These studies have led to the conclusion that since the specific absorbtion increases as the level of organization increases, interactions between macromolecules may be affected by ultrasound.

Hawley et al. (1963) have reported that exposure of degassed solutions of DNA to 0.98 MHz ultrasound at intensities of 25 to 31 W/cm^2 resulted in a decrease in the sedimentation coefficient from 32 to 16 which corresponds to a molecular weight change from 2.2×10^7 to 4×10^6. This change occurred within the first 15 seconds of exposure and little further degradation took place over a 2-minute irradiation suggesting that a limiting molecular weight had been reached. No cavitation (as evidenced by visible bubbles) or heating could be detected and the authors suggested that the degradation was due to viscous stresses in the molecule resulting from the density difference between DNA and water.

Irradiation of calf thymus DNA in vitro with 1 MHz focused ultrasound caused degradation of the DNA at 200 W/cm^2 with exposures of 10 minutes or longer (Coakley & Dunn, 1971, 1972). The authors stated that transient cavitation was insufficient to account for the breakage observed and suggested that the mechanism involved microstreaming. It is interesting that greater degradation occurred with the 200 W/cm^2 intensity (apparently in the absence of cavitation) than with an intensity of 515 W/cm^2 where cavitation was suspected as the mechanism involved.

Degradation of calf thymus or salmon sperm DNA was reported after exposure to 1 MHz ultrasound radiation for 3 minutes at intensities greater than 0.4 W/cm^2 (Hill et al., 1969). The molecular weight decreased with increasing ultrasound intensity up to 3 W/cm^2 and no further decrease occurred with intensities up to 8 W/cm^2 indicating that a limiting molecular weight had been reached. This terminal molecular weight was also obtained with a 5-minute exposure at 2.5 W/cm^2; longer times (up to 20 minutes) caused no further DNA degradation. These results with low intensity ultrasound could only be obtained if the exposure vessel were rotated during the experiment. The authors concluded that the mechanism for the ultrasound-induced DNA degradation involved stable cavitation in which the microbubbles were induced to move in a circular path by rotation of the vessel thus increasing their effective lifetimes.

Irradiation of calf thymus DNA with 1 MHz ultrasound resulted in considerable damage when the lengths of sonicated and control DNA samples were examined by electron microscopy after exposure to as little as 200 mW/cm^2 (Galperin-Lemaitre et al., 1975).

While there was no observable effect at 20 mW/cm^2, an intensity used in obstetrics, the authors pointed out that 200 mW/cm^2 is only 10 times this intensity and in obstetric diagnosis the ultrasound may be applied for several hours. It should be noted that some diagnostic pulse-echo devices may approach 200 mW/cm^2 (section 4.1).

Sonication of H. influenzae DNA in a tissue disrupter (MSE 3100 apparatus) at 20 kHz and an intensity of 32 W/cm^2 for 1 to 10 minutes resulted in a reduction in transforming activity with increasing time of sonication (Gouchet & Chevallier, 1971). The authors suggested that the reduced activity was the result of a molecular weight reduction, but could not demonstrate such a decrease. Furthermore, the frequency used (20 kHz) is much lower than the 1 to 10 MHz range generally used in medicine. However, a report by Wang (1977) showed that exposure of DNA isolated from a streptomycin-resistant strain of H. influenzae to 1 MHz ultrasound at an intensity of 5 W/cm^2 for 30 minutes caused a ten-fold inactivation of transforming activity as assayed by incubation with the wild type. No evidence of single or double strand breaks was found with exposure of either SV-40 or ϕx-174 DNA to ultrasound radiation. However, Wang (1977) stated that the exposure vessel used in these latter studies might be suspect because of the use of Parafilm but it is not clear why such use would lead to negative results.

Several studies have been performed using components of DNA and RNA such as nucleotide bases, nucleosides and nucleotides (Wang, 1977; Gupta & Wang, 1976; Wang & Gupta, 1977). Exposure of the bases to intensities of 3 and 5 W/cm^2 resulted in the production of sonoproducts with the order of reactivity being: thymine >uracil >cytosine>guanine>adenine. In the case of thymine, 50 percent had reacted after an exposure duration of 20 minutes at 5 W/cm^2. It was also found that nucleosides (base + sugar) and nucleotides (base + sugar + phosphate) were less reactive than their corresponding bases and thus it appears that the addition of a sugar or a phosphate group affords protection from sonolysis. Analysis of the sonoproducts obtained indicated that they were mainly glycols which the authors suggested were formed by reaction of the nucleotide bases with OH free radicals produced in water as a result of cavitation (Gupta & Wang, 1976). Similar results were obtained with pulsed ultrasound at a time average intensity of 3 W/cm^2 (Wang & Gupta, 1977). It was found that the extent of sonolysis was greater with the pulsed ultrasound (pulse durations of 20 µsec to 10 msec) than with continuous wave and that a greater effect was observed with the longer pulse widths.

Some studies have been performed to determine the effects of ultrasound on enzymes and on enzyme-catalyzed reactions in vitro. The enzymes studied included α-chymotrypsin, trypsin, aldolase, lactic dehydrogenase, and ribonuclease. The solutions were exposed from 0.12 seconds to 10 minutes with ultrasound frequencies of 1 to 27 MHz and intensities as high as 10^4 W/cm^2 (Coakley & Dunn, 1972). The investigators concluded that cavitation was necessary for enzyme degradation in vitro and that if these studies could be extrapolated to in vivo conditions it appears that proteins would be damaged only with very intense fields. In a more recent study (Kashkooli et al. 1980), degassed solutions of catalase and malate dehydrogenase were sonicated for 15-90 min with a vibrating wire driven at 20 kHz (tip displacement amplitudes varied from 15-26 µm). While catalase appeared to be unaffected by the ultrasound treatment, the enzyme activity of malate dehydrogenase decreased exponentially with increasing time of sonication. The authors concluded that the enzyme inactuation was due to acoustic microstreaming and was not a result of collapse cavitation or temperature elevation. Although these results suggest that enzymes can be damaged by a nonthermal and noncavitational mechanism, the low frequency used and the lack of conventional intensity values makes it difficult to evaluate this study with respect to medical ultrasound exposure. However, as pointed out earlier in this section since interactions between macromolecules such as proteins appear to be affected by ultrasound, it is possible that subcellular structures could be more labile to damage from low intensity ultrasound (Edmonds, 1972) than their component biomolecules.

A few reports have appeared in which the effect of ultrasound on isolated cell organelles was studied. Although no details of the irradiation were given, Hughes (1972) reported that ultrasound exposure of isolated mitochondria produced results which indicated that the

coupling of oxidative phosphorylation to respiration was very sensitive and could be altered at low shear gradients below those attributed to transient cavitation. He further hypothesized that even a 2 to 3 °C increase in temperature could be enough to cause effects with membrane systems and with enzymes involved in key control processes in vivo. Treatment of microsomes and ribosomes isolated from rat liver for 1 minute with a sonicating titanium probe resulted in the release of protein and RNA, a decrease in the amino acid incorporating ability, and an increase in phosphodiesterase activity (Von der Decken & Campbell, 1964; Campbell & Kernot, 1962). Electron microscopic analysis of the microsomes and ribosomes showed no obvious structural changes following the ultrasound exposure. However, the significance of the study is questionable because of the use of a sonicating probe with no indication of the frequency or intensity of the irradiation.

In summary, although solutions of macromolecules such as proteins and nucleic acids are capable of absorbing ultrasound in the megahertz frequency range, damage has been reported to occur usually only at high ultrasound intensities (25 W/cm^2 or greater) and generally as a result of cavitation. One report has indicated that DNA can be degraded in solution with low intensity (200 mW/cm^2) noncavitational ultrasound (Galperin-Lemaitre et al. 1975). However, it is not clear if this data can be extrapolated to the in vivo situation since the structure of DNA in solution bears little resemblance to its structure in vivo. In the latter case the DNA may be less sensitive to destruction by ultrasound since it is complexed with proteins which could protect the DNA from damage. On the other hand, the DNA in vivo is more structurally organized and therefore could be more sensitive to ultrasound-induced damage than it is in vitro. In this regard, when proteins and other macromolecules are assembled into subcellular structures, such as membranes, they may be more labile to damage by ultrasound at intensities and frequencies commonly used in medicine.

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5.3 CELLS

Studying cells in culture provides a means for examining the effects of different environmental factors, such as ultrasound, apart from the numerous biological variables operating in the intact animal. Moreover, studies directed towards elucidating the mechanism of action of a particular agent may be more readily performed and analyzed in cell cultures than in the whole animal.

A number of effects has been observed in mammalian cells after ultrasound exposure. These include cell lysis, cellular inactivation, altered growth properties, modification of cellular ultrastructure and macromolecular synthetic pathways, and chromosomal changes. This section will review the current information concerning such effects, with the exception of chromosomal changes which are discussed in section 5.4.2.2.

5.3.1 Effects on Macromolecular Synthesis and Ultrastructure

Both increases and decreases in the rates of DNA and protein synthesis have been reported to occur following exposure of cells in tissue culture to ultrasound. Stimulation in the rate of protein synthesis was observed 4 days after exposure of human fibroblasts to 3 MHz ultrasound for 5 minutes with intensities of 0.5 to 2.0 W/cm^2 (Harvey et al., 1975). With the continuous wave mode at 0.5 W/cm^2, total protein synthesis was increased by 20 percent while with the pulsed mode (pulse duration of 2 msec, duty factor of 0.2) at the same average intensity the increase was 30 percent greater than control values (Harvey et al., 1975; Webster et al., 1978). The stimulation appeared to be inversely related to the ultrasound frequency in the range of 1 to 5 MHz and the stimulation was abolished if the cells were pretreated with cortisol. The authors suggested that the increased protein synthesis observed was due to damage of the lysosomal and plasma membranes, possibly by a cavitational mechanism of action, since no ultrastructural changes occurred if the cells were exposed at elevated pressures. Increased DNA synthesis was observed at 1, 2, and 3 days following in vitro exposure of excised neonatal mouse tibiae to 1 MHz continuous wave ultrasound at 1.8 W/cm^2 (Elmer & Fleischer, 1974). However, no statistically significant differences were observed in either protein accumulation or in bone elongation when compared with the controls. The authors suggested that ultrasound can produce subtle changes in cellular processes without causing gross abnormalities, and that the changes observed may have been due to an effect on lysosomes.

Fung et al. (1978) exposed human lymphocytes to continuous wave ultrasound for 0 to 30 minutes using a standard fetal doppler unit. They measured the uptake of (^{3}H) thymidine over an 18-hour period 1 day after the ultrasound exposure and found two groups of individuals: (1) those whose lymphocytes showed significant stimulation of uptake at short exposure times (3 to 12 minute exposure) with a return to control values at longer exposure times (15 to 30 minute exposure) and (2) those whose lymphocytes exhibited no stimulatory effect at short exposure times but a significant reduction in uptake with 12 and 30 minute exposures. Stimulation of the lymphocytes with phytohemaglutinin (PHA) following the ultrasound exposure caused qualitatively similar results. The authors suggested that this biphasic effect could be due to a differential site of action of ultrasound on human lymphoblastic transformation in vitro. This effect could also be a result of differences in the response of T and B cells to ultrasound since the relative distributions of T and B cells between the two groups of individuals were not known.

Exposure of Pisum sativum root meristem cells to 2 MHz continuous wave ultrasound for 1 minute at an intensity of 30 W/cm^2 decreased the amounts of DNA, RNA, and protein synthesis (Miller et al., 1976). DNA synthesis remained depressed for 8 to 9 hours after irradiation while RNA and protein synthesis recovered after approximately 2.5 to 3 hours

post irradiation. The mitotic index (number of cells in mitosis/number of cells) was also depressed initially but recovered approximately 6 to 10 hours after sonication. The degree of initial depression increased with increasing intensity of the ultrasound in the range of 7.5 to 20 W/cm^2. These effects of ultrasound may be due to disruption of intracellular organization initially, followed by realignment of the affected constituents to their previous positions.

Incorporation of (^3H) thymidine and (^3H) deoxyuridine into DNA was decreased to a level of 54 percent and 42 percent of control values, respectively, following exposure of L-1210 cells to 2.22 MHz ultrasound for 10 minutes at a mean spatial intensity of 10 W/cm^2 (Kaufman & Kremkau, 1978). Addition of anticancer and antineoplastic drugs caused further reductions in incorporation. Injection of these treated (ultrasound + drug) leukemic cells into recipient hosts resulted in significant increases in host survival compared with those injected with cells treated with the drugs alone. The authors concluded that ultrasound causes a reversible injury to the cell which is not readily reversed in the presence of cytotoxic drugs and this results in a significant decrease in lethal potential of the leukemic cells. In other studies, exposure to a pulsed diagnostic ultrasound unit (1 MHz, 4 mW/cm^2, on time 10 minutes) induced a reversible 14 percent reduction in the rate of DNA synthesis in nonsynchronized HeLa cells (Prasad et al., 1976), and exposure of guinea pig skin to 3 MHz ultrasound for 30 seconds at 1.7 W/cm^2 led to altered sulfate metabolism (Wells, 1974).

Liebeskind et al. (1979a) reported that exposure of synchronized HeLa cells in culture to pulsed 2.5 MHz ultrasound at SATA intensities of 17 mW/cm^2 and 35.4 W/cm^2 SPTP intensities induced unscheduled non-S-phase (repair) DNA synthesis. Although this result suggested that the DNA had been damaged by the ultrasonic exposure, the authors could find no evidence for increased frequency of sister chromatid exchanges with this cell line. However, in another study Liebeskind et al. (1979b) found a small but significant increase in the frequency of sister chromatid exchanges following a 30-minute exposure of normal human lymphocytes to pulsed diagnostic ultrasound of frequency 2.0 MHz, 4.5 mW/cm^2 (SATA).

Ultrasonically induced functional alterations of the plasma membrane have been reported by a number of investigators. These alterations include increased permeability, decreased active transport, decreased nonmediated transport, and decreased electrophoretic mobility. For example, Bundy et al. (1978) have demonstrated a 5 percent decrease in nonmediated transport of leucine in avian erythrocytes following ultrasound exposure at 1 MHz and 0.6 W/cm^2. The authors suggested that the mechanism involved nonthermal and noncavitational acoustic microstreaming.

A reduction in the electrophoretic mobility of Ehrlich ascites tumor cells was found to be directly proportional to the square root of the ultrasonic frequency used in the range of 0.5 to 3.2 MHz (Taylor & Newman, 1972). This reduction in mobility was reported to be independent of the pulse length over the range of 20 μs to 10 ms (peak intensity was 10 W/cm^2; duty factor of 0.1, exposure time of 5 min). The change in mobility was presumably a result of alteration of the surface charge of the cells which the authors suggested was due to a nonthermal and noncavitational mechanism involving mechanical forces such as streaming and shearing. It is noteworthy that this effect could be shown with pulse lengths as short as 20 μs since such ultrasonically induced changes in the surface properties of tumor cells could render them less susceptible to removal by the immune response in vivo.

A mechanical stress mechanism of action was also suggested as the cause of an increase in the permeability of human erythyrocyte membranes to potassium ions which was observed following ultrasound exposures for 5 to 30 minutes (1 MHz, 0.5 to 3.0 W/cm^2) (Lota & Darling, 1955). A decrease in potassium content was reported to occur following insonation of rat thymocytes for 40 minutes using an ultrasonic therapy unit operated at 3 MHz and 2 W/cm^2 (Chapman et al., 1979). This decrease in intracellular potassium appeared to be

a result of both a decreased influx and an increased efflux and the authors proposed that the mechanism involved nonthermal and noncavitational acoustic microstreaming.

However, Lehmann and coworkers (1954 a&b) proposed a thermal mechanism for the membrane alterations which they observed following exposure of isolated frog skin to 1 MHz ultrasound. They used both the continuous wave (1.8 and 3 W/cm^2) and pulsed (1.8 W/cm^2, SATA, 30 ms pulses, 1:1 regime) modes and found an irreversible increase in membrane permeability, a decrease in the active transport of sodium ions, and an increase in the isoelectric point of the membrane. These same effects were observed with both the continuous wave and pulsed modes and could also be duplicated by increasing the temperature to 41 °C in the absence of ultrasound. The authors concluded that all of these changes were due to heat denaturation of membrane proteins.

Changes in the concentrations of membrane-associated cAMP and cGMP have profound effects on a great variety of cellular processes. However, no alterations in the amount of cAMP or cGMP could be detected following exposure of human amniotic cells or mouse peritoneal cells to continuous wave ultrasound for 2000 seconds at 1 MHz and 1 W/cm^2 (Glick et al., 1979).

Siegel et al. (1979) reported that dispersed cultured human cells seeded in plastic petri dishes showed significantly reduced cellular attachment after 0.5 minute of exposure to a 2.25 MHz pulse clinical diagnostic ultrasound source with a 19-mm-diameter transducer and a total power output of 1.76 mW. The SPTP and SATA intensities were not given. The human amniotic cell line JHA was found to be especially susceptible to the ultrasonic exposure. The authors suggested that such decreases in cellular attachment could affect in vivo implantation, morphogenesis and development.

Numerous reports have appeared describing ultrastructural damage to cells exposed to ultrasound. Suspensions of rat bone marrow cells irradiated with 0.8 MHz ultrasound for 1 minute at 1.5 W/cm^2 exhibited gross damage when examined by electron microscopy (EM) (Dunn & Coakley, 1972). It was suggested that high particle acceleration forces associated with the sound wave were responsible for the damage but a cavitational mechanism was also possible. Deformation, rotation, and fragmentation of nucleoli and nuclei and acoustic streaming of cytoplasm and nucleoplasm were observed when egg cells of marine invertebrates were subjected to ultrasound by means of a vibrating tip driven at 85 kHz (Wilson et al., 1966). Treatment of the protozoan Tetrahymena pyriformis with a vibrating needle caused the cell contents to move in a circular manner relative to the cell motion (Hughes & Nyborg, 1962). No bubble formation or cavitation could be detected by microscopic examination during the exposure. Microscopic observation of plant cells during the application of ultrasound by means of a vibrating tip (Mason Horn) operated at 80 to 90 kHz showed streaming, aggregation, and rotation of intracellular particles and organelles (Nyborg et al., 1977). The authors suggested that these motions were due to radiation force and acoustic streaming. Changes in calcium permeability of muscle cells and interference with cell division in Arbacia egg cells have also been reported to occur as biological responses of cells to these types of motions (Dunn & Coakley, 1972). However, irradiation of mouse lymphoma cells suspended in a gel for 5 minutes at 15 W/cm^2 caused no ill effects (Dunn & Coakley, 1972). In this case very little temperature rise occurred and cavitation was supressed by the gel medium.

Human fibroblasts were irradiated with pulsed 3 MHz ultrasound at 0.5 W/cm^2 with a duty factor of 0.2 and examined by EM (Harvey et al., 1975). A variety of ultrastructural changes were observed including more free ribosomes, increased dilatation of the rough endoplasmic reticulum, increased damage to mitochondria and to lysosomal membranes, and more cytoplasmic vacuolation. As noted earlier in this section, the exposed cells also exhibited increased protein synthesis when assayed 4 days after irradiation. The authors suggested that these changes were due to cavitation and that the stimulation in protein synthesis could have resulted from: damage to the lysosomal membrane leading to an increased pool of catabolites, a decline in pH and an increase in osmolarity resulting from the released hydrolytic enzymes. These alterations could initiate protein synthesis by

derepression of DNA or by enlargement of the precursor pool. Exposure of HeLa cells to 0.75 MHz ultrasound at an intensity of 0.9 W/cm^2 for 20 to 120 seconds caused many ultrastructural changes such as slits in the cells, holes in the nuclear membranes, separation of the inner and outer nuclear membranes, increase in cell debris, imploded mitochondria, and lesions of the endoplasmic reticulum (Watmough et al., 1977). The results suggested that some of the damage such as rupture of the nuclear and plasma membranes may have been due to shearing stresses resulting from microstreaming around resonant microbubbles. However, invagination of the nuclear membrane and lesions of the endoplasmic reticulum appeared to result from a more localized mechanism of damage such as degassing within the cells leading to localized pressure changes.

Roseboro (1977) observed imperfections in cell surface morphology of human lymphocytes and constricted and spherical mitochondria and small nuclei in HeLa cells examined by EM following exposure of these cells to 65 kHz ultrasound at an intensity of 0.7 W/cm^2. He suggested that these effects were due to microstreaming resulting from stable cavitation. Two types of reactions were observed in studies with root tips of Allium cepa which were exposed to 0.8 or 1 MHz ultrasound for 2 minutes at intensities of 10 to 33 W/cm^2 (Lehmann et al., 1954c). In the first type of reaction, the greatest damage was induced in cells in the center of the root tip and in some cases cell walls and nuclei disappeared leaving a mass of debris. In the second type of reaction, the damage was more uniformly distributed over the entire root tip and included shrinkage and vacuolization of the cytoplasm and nuclei. Degassing within the tissue was suggested as the mechanism for the damage produced in the first type of reaction since it was prevented by increasing the pressure to 6.7 atm. However, it was not known if the degassing occurred within the cells or in the intracellular spaces. The second type of reaction appeared to result from a thermal mechanism of action since it could be reproduced by increasing the bath temperature to 41 °C in the absence of ultrasound.

Other studies with human lymphocytes and Erlich ascites carcinoma cells suggested a possible disturbance of the mitotic spindle at metaphase following ultrasound exposure (Schnitzler, 1972) and Clarke & Hill (1969) have reported an increased susceptibility to ultrasonic disintegration during mitosis in L5178Y cells. It was suggested that cells are particularly susceptible to damage by ultrasound during mitosis since major changes in the cell membrane and in internal structure occur during this phase of the cell cycle.

El'Piner (1972) has observed that ultrasound can cause disruptions in the spatial relationships of macromolecules in cell membranes and has postulated that this will cause exposure of new active centers and functional groups. For example, increased peroxidase activity was observed following exposure of potato tubers to ultrasound. In addition, disturbance of the ultrastructure of cell surface layers by ultrasound can lead to disruption in the exchange of nutrients into and out of the cell. This is supported by the observation that exposure of bacteria or erythrocytes to apparently noncavitational ultrasound caused leakage of cell contents such as protein into the medium (Hughes, 1972; Hughes & Nyborg, 1962), as well as by the reports cited earlier of changes in the permeability of eukaryotic cell membranes. Furthermore, Martins (1971) reported that scanning electron micrographs of M3-1 cells exposed to 1 MHz ultrasound at 1.0 and 0.25 W/cm^2 show a characteristic bumpy outer surface compared with the smooth outer surface of unexposed cells. Liebeskind et al. (1981a) have also reported morphological changes in the surface characteristics of exposed cultured mouse 3T3 cells many generations after a 30-minute exposure to pulse diagnostic ultrasound with SATA intensities of 15 mW/cm^2 and SPTP intensities of 35.4 W/cm^2. A majority of the exposed cells exhibited a large increase in the number of surface microvilli compared with the control cells which could represent an early phase in the progression toward the transformed state. In addition, both 3T3 cells and rat peritoneal fluid cells insonated under the aforementioned conditions exhibited many ultrastructural alterations such as clustering of perichromatin granules, invagination of the cytoplasm into the nuclear domain, separation of the nuclear membrane, change in the arrangement of intermediate filaments and microtubules, and engorgement of rough endoplasmic reticulum cisternae (Liebeskind et al. 1981b). Furthermore, the exposed 3T3 cells spread poorly, were less contact inhibited, and their entire surface was in motion; this behavior persisted

for at least 15 generations during all stages of the cell cycle. The authors suggested that alterations in the function of microtubules and microfilaments following exposure to low-level pulsed ultrasound could account for many, if not all, of the observed results.

In summary, irradiation of cells in culture with ultrasound in the therapeutic and diagnostic intensity ranges can cause changes in ultrastructure which may lead to disruptions in macromolecular synthetic pathways. Certain structural components seem especially labile to damage; these include the nuclear, lysosomal, and plasma membranes, the cytoskeleton (microtubules and microfilaments) and the endoplasmic reticulum. Both ultrastructural and functional changes of the plasma membrane have been reported to occur following exposure to relatively low intensity ultrasound. Because of the importance of the cell surface in immune determination, receptor topography, carrier systems, cell-cell recognition, etc., such changes could have quite important ramifications in vivo. It also appears that release of hydrolytic enzymes from damaged lysosomes could lead to derepression of DNA and induction of protein synthesis as well as to increased DNA synthesis. Increased DNA and protein synthesis could also be a result of repair processes. On the other hand, decreased DNA synthesis could be due to a decrease in the transport of the nucleotide precursors resulting from damage to the plasma membrane, and disruption of the endoplasmic reticulum could cause a decrease in protein synthesis. Although cavitation appears to be the mechanism for many of the ultrasonically induced structural changes, it seems possible that some of these effects could be due to noncavitational mechanical stress and/or to temperature elevation.

5.3.2 Effect of Ultrasound on Mammalian Cell Survival and Proliferation

Many studies concerning cellular effects of ultrasound have utilized qualitative biological end points such as cell lysis or morphological changes in cell structure. From the mid-1970's, however, investigators began to focus their attention on quantifiable biological parameters such as cell survival and proliferative capacity. Lysis of mouse lymphoma cells in suspension at ultrasound frequencies and intensities used in clinical medicine has been documented and correlated with acoustic cavitation (Clarke & Hill, 1970; Coakley et al., 1971). Moreover, it has been suggested that intact cells surviving ultrasound exposure remain unaffected in terms of subsequent growth rate and proliferation (Clarke & Hill, 1969). More recently, however, studies have shown that many of the intact nonlysed cells remaining after ultrasound exposure of mammalian cells in suspension are nonviable as determined by both vital dye exclusion and colony-forming ability (Kaufman, et al., 1977). Exposure of HeLa cells for 2 minutes to continuous wave ultrasound at a frequency of 1 MHz resulted in a threshold for cell lysis at approximately 1 W/cm^2 with the maximal effect at an intensity of 10 W/cm^2 (Kaufman et al., 1977). The intensity versus response curve for cell viability was similar to that for cell lysis with respect to threshold intensity and intensity for maximal effect. Both parameters decreased at intensities higher than 10 W/cm^2. Approximately 25 percent of the cells were intact after a 5-minute exposure at an intensity of 10 W/cm^2. However, 60 percent of the intact cells were nonviable as determined by vital dye exclusion. Similar exposure of Chinese hamster ovary (CHO) cells resulted in reduced viability as determined by colony-forming ability (Kaufman et al., 1977). Viability determined by this method was comparable to that determined by vital dye exclusion. Appropriate controls showed that neither temperature nor a medium component was responsible for the observed effects. The authors attribute cell lysis and inactivation to ultrasound-induced cavitation.

The formation of abnormally large so-called giant cells occurs after x irradiation of cell cultures and is correlated with loss of reproductive capacity of cells surviving irradiation. It was noted in the aforementioned studies that colonies formed from sonicated cells contained fewer cells and a higher frequency of giant cells than colonies formed from appropriate controls, i.e., unexposed or sham exposed (Kaufman et al., 1977). This observation was extended by further studies which showed that exposure of CHO cells to continuous wave ultrasound at a frequency of 1 MHz, and intensity of 5 W/cm^2 for 5 minutes resulted in sonicated colonies which contained fewer cells and more giant cells than corresponding controls (Miller et al., 1978). The frequency of giant cells was

approximately 2.5 times higher in colonies arising from sonicated cells. Characteristics of giant cells varied from abnormally large cells with one large nucleus or several smaller nuclei to cells containing protoplasmic bridges, suggestive of cell fusion. Giant cell formation after ultrasound exposure has been interpreted as a loss of reproductive capacity (Miller et al., 1978), reminiscent of that previously observed with x irradiation.

Recent studies with V-79 Chinese hamster cells have investigated the delayed effects of ultrasound exposure. It has been observed that increasing lysis occurred in mitotic cells as the exposure duration of ultrasound at a frequency of 1 MHz and intensity of 3 W/cm^2 was increased from 0.1 to 10 minutes (Ciaravino & Miller, 1978). Exposures greater than 0.1 minute (1 to 10 minutes) revealed an approximate 20 percent decrease in growth rate within 4 hours post exposure as measured by colony-forming ability. Growth rate was not affected after 4 hours and up to 50 hours post exposure. However, (^{14}C) TdR labeling studies showed that in addition to the cell lysis observed immediately after ultrasound exposure, 16 percent of the intact post sonicated cells lysed within an additional 24 hours. Further studies have shown that the surviving intact cells returned to their normal growth rate by approximately 36 hours after exposure (Kaufman & Miller, 1978). Such studies seem to indicate that ultrasound produces both acute and delayed effects and that a recovery phenomenon may be operating.

It is interesting that, in the aforementioned studies, exposures to ultrasound at intensities greater than 5 to 10 W/cm^2 decreased the effects observed. Further work has shown that the presence of cysteamine, a free radical scavenger, in V-79 cells also resulted in a dose reduction at intensities of 20 and 30 W/cm^2 (Fu & Miller, 1978).

Data obtained from different studies may vary quantitatively depending on the mode of ultrasound exposure, i.e., continuous versus pulsed. Mammalian cell survival, as measured by colony-forming ability, was decreased following exposure to continuous ultrasound at a frequency of 1 MHz and intensities of 0.25 to 4.0 W/cm^2 (Martins, 1971). Although the shapes of the survival curves were similar, survival was dependent on intensity, i.e., survival decreased as the intensity was increased. A threshold intensity was apparent, since effects were observed only at intensities above 0.125 W/cm^2. Although slight differences in survival were observed for the different cell lines examined, a similar survival pattern in response to ultrasound exposure was noted for both the human and Chinese hamster cell lines (Martins, 1971). The colony-forming ability of Chinese hamster ovary cells (HA-1) was reduced 10 percent at a frequency of 1 MHz and intensity of 2 W/cm^2 pulsed exposure (Li et al., 1977) as compared to 40 percent with continuous exposure (Miller et al. 1978). Increasing the temperature from 37 °C to 43 °C reduced the colony-forming ability 10 to 50 percent (Li et al., 1977). Thus, although cell lysis was temperature-independent, cell inactivation as measured by reduction in plating efficiency was temperature-dependent (Li et al., 1977). Conversely, exposure of Chinese hamster lung cells (CHLF) to continuous and pulsed ultrasound at a frequency of 1 MHz had no effect on survival as measured by colony forming ability (Bleaney et al., 1972). When the temperature of the cell cultures remained below 45 °C, no decrease in survival was observed with intensities up to 8.8 W/cm^2 for continuous radiation for 60 minutes, or with peak intensities of 15.0 W/cm^2 of pulsed radiation (Bleaney et al., 1972). However, as observed in the previous study (Li et al., 1977), it was concluded that cell damage as measured by colony-forming ability was due to excessive heating and not directly to the effect of ultrasound (Bleaney et al., 1972). Based on such observations, the use of ultrasound as an adjunct to x radiation in tumor therapy has been suggested (Li et al., 1977). That ultrasound may be a useful therapeutic tool is further suggested by studies showing that lymphocytes are more sensitive to ultrasound exposure than red blood cells (Roseboro et al., 1978). This raises the possibility of ultrasound use in conditions where selective destruction of lymphocytes is desirable.

In addition to studies of cell lysis, inactivation, and proliferative capacity, recent investigations have shown that ultrasound exposure tranforms the normally contact-inhibited mouse C3H 10T-1/2 cells into morphologically transformed clones which lost contact-inhibited growth (Liebeskind et al., 1979a). This is similar to the effects of

ultraviolet (UV) and x irradiation as well as chemical carcinogen-induced oncogenic cell transformation.

Further studies are warranted to confirm the observations described above in a number of the mammalian cells. Certainly the potential use of ultrasound as a therapeutic modality either alone or in combination with x rays requires that further studies be performed in appropriate animal model systems. Further quantitative dose-response data of cell lysis and inactivation with respect to the ultrasound mode, i.e., continuous versus pulsed, should be obtained. Of importance in cell culture studies is the investigation of methods which would minimize or eliminate the problem of cavitation. It has been suggested that suspension of cells in a gel medium may be helpful in this regard.

Summary for Section 5.3

Exposure of different types of cultured mammalian cells to ultrasound in the diagnostic and therapeutic intensity ranges affects cell survival and proliferation. This is manifested as cell lysis, or cell inactivation and reduction in cell growth rate as determined by vital dye exclusion or colony-forming ability. Giant cell formation, indicative of loss in reproductive capacity, has also been observed. Such effects have been noted both immediately and at different times after ultrasound exposure. The results obtained vary depending on the mode of irradiation, i.e., continuous versus pulsed. Cell survival decreases as intensity increases and a threshold is apparent when cultures are irradiated continuously. There is less reduction in cell survival with pulsed exposure. In addition, to the effects just described, studies have shown that ultrasound exposure transforms normal contact-inhabited cells into morphologically transformed cells, an effect previously observed with other types of radiation and chemical agents.

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5.4 EFFECTS ON ANIMALS

5.4.1 Developmental Effects

To date much of the work on the effect of ultrasound on animal development has occurred in three major organisms, Drosophila, mice, and rats with some work being done on others, such as the amoeba.

5.4.1.1 Drosophila

The earlier efforts involved the exposure of Drosophila melanogaster (D.m.) eggs, larva, and prepupa. Fritz-Niggli & Boni (1950) induced developmental abnormalities by exposing these stages to 800 kHz ultrasonic vibrations at an intensity of 1.75 W/cm^2. They demonstrated that the egg, larva, and prepupa were sensitive to ultrasonic vibrations, with the prepupa being most sensitive. They also reported a delayed death phenomenon in which exposed organisms died at a later stage of development. Selman & Counce (1953) found abnormally developed embryos following the exposure of D.m. to 1 MHz continuous wave ultrasound during the syncytial blastoderm stages (2 hours post laying at 25 °C) at intensities of 0.3 to 0.5 W/cm^2 for 30 seconds. Counce & Selman (1955) demonstrated that irradiation of D.m. eggs at the syncytial blastoderm stage at intensities of 0.3 to 0.5 W/cm^2 resulted in a high proportion of developmental abnormalities which they classified into five categories: (1) immediate death; (2) cell proliferation with differentiation; (3) differentiation without organization; (4) abnormal organization; and (5) small abnormalities in organization. These early studies provided little information on exposure conditions but gave an indication that embryonic exposure to ultrasound resulted in abnormal development.

Pay et al. (1978) exposed D.m. pupae (122 ± 2 hours post fertilization) to 1 MHz (continuous wave) ultrasound at intensities of 0.2 to 4.0 W/cm^2 for 10 minutes, and observed abnormal eye pigmentation and abnormal eye, head, and thorax development in non-eclosed adult flies just prior to emergence. These adults had continued to develop to a point just before eclosion (emergence of the adult) as indicated by the development of the abdominal bristles. These effects could have been a result of ultrasonic damage to the head and mouth region of the insect. This could have resulted in the insect's inability to engulf air (from inside the pupal case) and break off the opreculum to emerge. This work supported Fritz-Niggli's observation of delayed death in sonicated prepupa.

Pizzerello et al. (1978) obtained "small flies" that developed from D.m. larvae and pupae exposed for 2.5 minutes to 2.25 MHz pulsed ultrasound at an intensity of 1.5 mW/cm^2. Ultrasonication of D.m. during the third instar larval or pupal stage was highly lethal. However, the emerging flies from these groups were reported to be normal in appearance but were very small. However, the investigators did not describe either the dimensions of the small flies or the number of organisms studied.

In an attempt to repeat the work of Pizzarello et al. (1978), Child et al. (1980a) did not find the development of "miniature flies" upon exposure of D.m. larvae to SATA intensities of 1.5 mW/cm^2 pulse ultrasound. However, they did find that when the temporal peak power was increased by a factor of 10, approximately one-fourth of the larvae failed to survive to the imago stage. In another study, Child et al. (1980b) reported that when exposing D.m. to microsecond pulses of 2 MHz ultrasound, the effects depended strongly on the temporal peak intensity with killing observed at SPTP intensities above 10 W/cm^2. They reported that the temporal average intensity appeared to be a poorer predictor for these biological effects than the peak intensity although marked decreases in the survival rates were observed at SATA intensities as low as 3 mW/cm^2.

Carstensen & Child (1980) exposed D.m. eggs to 1 MHz ultrasound at an intensity of 5 W/cm^2 for 30 seconds, killing 70 percent of the exposed eggs. The absorption coefficient of the eggs was 5 nepers cm^2 and the maximum temperature increments less than 3 °C. The authors claimed this value was too low for the observed killing to be attributed to heating.

The work on the effect of ultrasound on D.m. development is very sketchy and incomplete at best. One experiment cannot be directly compared to another, but there seems to be somewhat of an underlying consistency; that is, exposure of pre-imago D.m. to the reported level of ultrasound resulted in some types of abnormal development in the exposed organism.

5.4.1.2 Mice

5.4.1.2.1 Pulsed Ultrasound Effects. The results of the work with mice are less consistent than the results with Drosophila. Fry et al. (1977) (using focused beam ultrasound - 6 dB beam width of 2 mm) exposed LAF/J mouse testicles, nonpregnant mouse ovaries, and pregnant mouse uteri on the 8th day of gestation to pulsed ultrasound. The male testicles were bilaterally irradiated as were the nonpregnant ovaries. The nonpregnant females had the hair removed from the entire body. The pregnant females were irradiated transcutaneously. These mice were exposed to spatial peak, temporal peak intensities of 567 to 2018 W/cm^2, 1000 Hz pulse repetition frequency, ultrasound frequencies of 1.110 and 1.364 MHz, pulse widths of 15 to 125 µsec, and exposure time of 20 seconds. Although the pulse widths are somewhat greater than found in diagnostic units, a few diagnostic units have been measured with outputs of greater than 1000 W/cm^2 (section 4.1). Thirty days post exposure, the males, whose testicles were exposed, were placed with two females. The offspring that resulted were examined through three litters. For the first two litters, only litter size was examined. The third litter was removed by Caesarian section on day 18 post conception and examined for 29 types of anomalies. There was no statistically significant effect on weight, resorption, or runts, but there was a statistically significant reduction in litter size. This reduction tended to be associated with the right uterine horn. There was a significant increase in anomalies in the offspring of males exposed to 25 and 50 µsec pulse widths. Thirty days post exposure, each nonpregnant female was placed with a male and mated for the production of two litters. For the first litter, only litter size was examined. The second litter was removed by Caesarian section on day 18 post conception. There was no significant change in weight, late resorption, or runts, but there was a significant increase in litter size. There was no statistically significant increase in anomalies. The fetuses of the 8-day pregnant female exposed group were examined 10 days post exposure after removal by Caesarian section. There was a significant increase in types of anomalies in the irradiated group when compared to the controls. Anomalies were directly related to pulse widths, beginning at 25 µsec.

Warwick et al. (1970) used pulsed ultrasound at somewhat lower intensities than Fry. Warwick used intensities up to a peak 490 W/cm^2 (temporal average intensity of 27 W/cm^2) at frequencies of 1, 2, and 3 MHz to expose pregnant S.A.S.I.C.L. female mice on day 1 through day 5 of pregnancy. The mice were killed on either the 18th or 19th day of pregnancy. The fetuses were removed and examined for variations in weight. The uteri were examined for resorptions and dead fetuses. Of 297 exposed mice and 179 controls averaged in several treatment groups, there was no statistically significant variation in fetal weight, litter size, or incidence of resorption.

Woodward et al. (1970), in what appeared to be a repeat of Warwick's work using pulsed ultrasound of 1 to 3 MHz frequency and peak intensities up to 490 W/cm^2, exposed abdominally shaved pregnant females on 5 successive days. When 223 exposed mice and 132 controls were analyzed for mean litter size, mean number of resorptions, and mean number of abnormalities, no significant differences were found.

Hara et al. (1977) exposed abdominally shaved nonanesthetized ICR strain pregnant mice to 2 MHz pulsed ultrasound for 5 minutes on the 8th day of gestation. The pulse width was

180 μsec and the repetition frequency was 150 Hz. The total body weight increase of the pregnant mothers from the day of exposure to the 18th day of gestation was significantly lower for groups exposed to temporal average intensities of 50 mW/cm^2 and 600 mW/cm^2. For the 600 mW/cm^2 temporal average exposure, the corresponding temporal peak intensity was 22 W/cm^2. In the group exposed to pulsed ultrasound with an average intensity of 600 mW/cm^2, a higher incidence of fetal malformations was found. Temperature measurement on mice irradiated with pulsed 600 mW/cm^2 ultrasound showed a temperature rise during the 5-minute irradiation of about 3 °C (from 37.5 to 40.5 °C) which began decreasing immediately after irradiation stopped.

Smyth (1966) using pulsed ultrasound at 2.25 MHz at an intensity of 10 mW/cm^2 exposed lightly anesthetized 52-day-old male and 51-day-old female Charles River CD HaM/ICR mice. Testes of the males and right ovaries of the females were exposed 10 minutes each day for the 5 days preceding mating and 10 minutes each day for 10 days during the mating period. Exposures continued throughout gestation until 2 days before delivery, for an average of 17 days. For 348 offspring of exposed mice, no congenital abnormalities were observed. Of 391 second generation offspring, no abnormalities were found.

5.4.1.2.2 Continuous Wave Ultrasound Effects. Stolzenberg et al. (1980a) exposed S.W. mice on days 1, 8, and 13 post conception to continuous wave 2 MHz ultrasound at an intensity of 1 W/cm^2 beginning at a temperature of 37 °C. A significant reduction in mean body weight gain was observed for mice exposed on day 8 for 160 seconds and those exposed on day 13 for 100 seconds; also a significant reduction in mean fetal weights for mice exposed on day 1 for 180 seconds and days 8 and 13 for 140 seconds was observed. All exposures showed uterine temperature raised above 43 °C suggestive of a thermal effect.

In a later study, Stolzenberg et al. (1980b) exposed S.W. mice on day 8 post conception to continuous wave ultrasound of a frequency of 2 MHz and at intensities of 0.5 and 1 W/cm^2. Significant mean uterine weight decreases were observed on day 25 for mice exposed in utero to 0.5 W/cm^2 for 140 seconds and 1 W/cm^2 for 60 seconds. The authors suggest this is an indication of delay or impairment of maturation.

Torbit et al. (1978) exposed the right or left ovary of virgin S.W. mice to focused continuous wave ultrasound at a spatial peak intensity of 43 W/cm^2 and a frequency of 2.0 MHz for 5 and 10 seconds. Unlike Stolzenberg, Torbit exposed the ovaries outside the body cavity. Immediately after exposure, the ovaries were reinserted in the abdomen. At 36 hours post exposure, ovulation was induced with exogenous gonadotrophins. Forty-eight hours later these females were mated. On day 11 of pregnancy, the female mice were sacrificed and examined. In both exposure groups, there was a significant decrease in mean number of embryos, when compared to the nonirradiated control ovaries. However, the mean number of embryos was higher in the 5-second group than for the 10-second group.

O'Brien (1976) observed a significant reduction in average weight for fetuses of anesthetized, abdominally shaved CF1 mice exposed on the 8th day of gestation to 1 MHz continuous wave ultrasound at intensities of 0.5 to 5.5 W/cm^2, with time varying from 10 to 300 seconds. Fetuses were removed by laparotomy on the 18th day and examined for variations in weight. Of the 2,866 fetuses from 273 litters examined, all exposed groups combined showed a significant average weight reduction, but O'Brien did not indicate which individual exposure groups showed significance.

Shoji et al. (1971), used continuous wave ultrasound and exposed DHS mice on the 9th day of gestation to 2.25 MHz ultrasound at an intensity of 40 mW/cm^2 for 5 hours. Fetuses showed no "growth-inhibiting" effects on body weight deviations when they were examined on day 18 of gestation. However, when Shimizu & Shoji (1973) repeated this experiment using DHS and A/He strain mice, a significant increase in frequency of external anomalies was seen in the A/He mice but not in the DHS strain. A significant increase in skeletal abnormalities was also reported for both strains.

Stratmeyer et al. (1977) showed a significant weight increase in fetuses of anesthetized, abdominally shaved CF1 mice that were exposed on the 10th day of gestation to 1 MHz continuous wave ultrasound at intensities of 0.25 and 0.80 W/cm^2 for 120 seconds. Fetuses were examined on day 18, 21, 36, and 51 post conception. For mice examined on days 21, 36, and 51 post conception, the 0.25 W/cm^2 group was significantly heavier than the shams. This contrasts with the results of O'Brien which showed a significant combined average weight reduction. Perhaps the difference lies in the fact that O'Brien exposed on day 8 of gestation and Stratmeyer exposed on day 10 or that O'Brien examined fetal weights and Stratmeyer examined weights of surviving pups. Also, O'Brien's exposure intensities did not reach the low levels of Stratmeyer's 0.25 W/cm^2 group which showed significance.

Stratmeyer's results also contrast with those of Stolzenberg who found a significant average weight reduction for fetuses exposed in utero to 1 W/cm^2 on day 13 of gestation. When comparing these contracts, it should be kept in mind that both O'Brien and Stolzenberg examined fetal weights, and Stratmeyer examined the weight of surviving pups. These results suggest that the production of observable effects may be a function not only of exposure intensity, but also of the day of gestation on which the organism is exposed.

In a subsequent experiment, Stratmeyer et al. (1979) exposed ICR mice on the 10th DPC of the second pregnancy to 1 MHz ultrasound at intensities of 0.075 W/cm^2 and 0.750 W/cm^2. The mice had their abdomens shaved and were anesthetized. They were sacrificed at 6 months and 2,603 pups were analyzed for body weight, brain, liver, heart, and kidney weight. There were significant variations between exposed and sham of some groups, although in some instances there was a lack of a meaningful trend with increasing exposure. However, in the case of the males, the weights of the exposed groups were consistantly lower than those of the unexposed groups, with the exception of the spleen weights. The 0.750 W/cm^2 group exhibited the highest spleen weight.

Shimizu (1978) reported a tenfold increase in abnormal fetuses for DHS strain mice exposed to 1.4 W/cm^2 continuous wave ultrasound for 5 minutes at a frequency of "approximately" 2 MHz. No increase in abnormal fetuses was observed at exposure levels of 0.5 and 0.75 W/cm^2. Tachibana et al. (1977) using 2.3 MHz continuous wave ultrasound exposed CF1 mice once daily on the 7th to 13th days of gestation at intensities of 80 and 100 mW/cm^2. The 7th-13th day is generally recognized to be the stage of organ development. A statistically significant difference was observed in fetal body weight reduction and macerated fetuses depending on the intensity and duration of the exposure.

Curto (1975) exposed anesthetized, abdominally shaved CF1 mice in utero to 1 MHz continuous wave ultrasound on the 13th day of gestation at intensities of 0.125, 0.25, and 0.5 W/cm^2 for 3 minutes. She observed an increased mortality rate on day 21 post weaning. In a study using a similar intensity (0.44 W/cm^2) and exposure time to those used by Curto, Edmonds et al. (1979) did not find any effects on neonatal mortality in mice exposed in utero. They used a frequency of 2 MHz, FW mice, and exposed on day 8 of gestation. Hara et al. (1977) observed a significant lower body weight increase of pregnant mothers and an increase in fetal malformations when pregnant ICR strain mice were exposed to continuous wave 2 MHz ultrasound at an intensity of 2 W/cm^2 for 5 minutes on the 8th day of gestation. Temperature measurement on mice irradiated with 2 W/cm^2 showed the uterine temperature raised to 41.5 °C during the 5-minute exposure which began decreasing immediately after irradiation stopped.

5.4.1.3 Rats

Sikov & Hildebrand (1976) exposed anesthetized rats in utero to intensities of 2.8 to 32.4 W/cm^2 focused beam continuous wave 3.2 MHz ultrasound on the 9th day of gestation. The fetuses were examined on day 20 of gestation. They reported that the dose response indicated a threshold intensity for prenatal mortality of 3 W/cm^2. The size of the fetus did not vary significantly from the controls at any exposure level. When Sikov et al. (1977) exposed rat fetuses in utero on day 15 of gestation to intensities of 0.01 to 1.0 W/cm^2

continuous wave ultrasound for 5-minute exposures at a frequency of 0.93 MHz, they found an increased incidence of prenatal mortality and delayed neuromuscular development. They found no evidence of increased postnatal mortality or reduced growth rate. These results contrast with the findings of Curto in which the entire abdomen was exposed instead of the uterus; these contrasting results may be attributed to the differences in the species exposed or differences in developmental stage at exposure. Sikov & Hildebrand (1977) exposed rat embryos in utero on the 9th day of gestation to focused beam ultrasound for either 5 or 15 minutes at frequencies of 0.71 MHz and 3.2 MHz continuous wave ultrasound or 2.5 MHz pulsed. In the continuous wave exposed groups, they observed an increased prenatal death above a threshold of 3 W/cm^2, and multiple developmental malformations at higher intensities. The major effect of the pulsed exposure was an increased incidence of gross and microscopic heart abnormalities.

Sikov and Hildebrand (1977) in a subsequent study observed cardiac defects in 9-day rat embryos exposed to 2.5 MHz pulsed ultrasound ranging from peak intensities of 15 W/cm^2 to 410 W/cm^2. They also indicated (as has Child (1980) using D.m. larvae) that the incidence of effects were more related to peak power than average power.

Pizzarello et al. (1978) exposed rats in utero to pulsed ultrasound for 5 minutes on days 3, 5, 6, and 15 at SATA intensities of 1.5 mW/cm^2 at a frequency of 2.25 MHz. The SPTP intensity was not reported. They observed no gross developmental abnormalities when the embryos were examined on day 17 of gestation. They reported that fetuses exposed on day 3, 5, or 6 were consistently smaller, and weighed less, but exposure on day 15 produced normal-sized fetuses. Pizzarello also found a threefold increase in resorption rates, when compared to the controls. However, no statistical analysis was presented and the number of animals examined was not given.

McClain et al. (1972) exposed pregnant rats to nonfocused continuous wave 2.5 MHz ultrasound at intensities of 10 mW/cm^2 for 0.5 or 2 hours on either days 8, 9, 10, 11, 12, and 13 of gestation. On day 20 of gestation, Caesarian section was used to examine for live, dead, and resorbed fetuses. Fetuses were examined for gross abnormalities and weight deviations. No significant differences were observed in body weight, viability, death, litter size, implantation, or skeletal or soft tissue abnormalities. There was a slight increase (nonsignificant) in resorption for animals treated for 0.5 hour on days 11, 12, and 13 of gestation and for 2 hours on days 8, 9 and 10; there was also a slight increase (nonsignificant) in skeletal variations in animals exposed for 0.5 hour on days 8, 9, 10, 11, 12, and 13.

Garrison et al. (1973) exposed the ovaries of pregnant Holtzman rats to temporal peak intensities of 10 W/cm^2 and 100 W/cm^2 through the body wall for 10 minutes, and 100 W/cm^2 directly to the surgically exposed ovary for 10 minutes. The pulsed exposure occurred at a frequency of 1.9 MHz on day 8 of gestation. All animals were sacrificed on either day 15 or day 17 of gestation; no significant effect on the percentage of resorptions, dead or live fetuses, or gross abnormalities was observed.

5.4.1.4 Amoebae

Brown & Coakley (1975) exposed a gel suspension of the amoebae (Acanthamoeba castellanii) to 1 MHz continuous and pulsed ultrasound at an intensity that had resulted in tissue lesions in higher animals (20 W/cm^2 for 5 minutes) and did not observe any effects on growth or cell division. The cells were exposed at an average temperature of "around 10 °C." This is considerably lower than the temperatures at which tissue was exposed by other researchers, and may account for Brown not observing effects that have been observed by others at similar intensities.

Summary for Section 5.4.1

The reported effects of ultrasound on animal development are confusing and in most cases impossible to compare directly owing, in part, to differences in the organism used, the stage of development at the time of exposure, and the exposure parameters. To make any hazard evaluations or set any rational standards, considerably more research is needed. The published work shows that if the intensity is sufficiently high, lethality or some type of anatomical abnormality will result in certain organisms. More work is needed to determine the temperature and temperature rise in the exposed organism. Most work to date concerning ultrasonic effects on animal development has included little or no temperature information in the exposed organism or in the general area of exposure. This is particularly important with continuous wave exposures in which heat may have more of an opportunity to build up than with short duration pulse exposures.

Ultrasound is known to raise the temperature of biological samples in which it is absorbed. The effects of exposure at higher intensities (Fry, 1977; O'Brien, 1976; Stolzenberg et al., 1980a; Torbit et al., 1978; Sikov, 1977; etc.) may be due to hyperthermia. Stolzenberg recorded a temperature rise in the uterus to 44 °C when exposing virgin mice at 1.0 W/cm^2 average intensity; this is considerably lower than the intensities used by most workers mentioned above. Effects of hyperthermia in rats and mice depend on the time and duration of exposure and include fetal resorption, retardation of growth, exencephaly, defects of the tail, limbs, toes, and palate (Lele, 1975). In regards to the work at lower intensity (Pizzarello et al., 1978; Curto, 1975; and Pay et al., 1978; etc.), the mechanism of action is unknown. The observed effects may be the result of cavitation, stress, some unknown parameter, or a combination of parameters.

There are indications that with pulsed ultrasound exposures it may be the peak power that is the determining factor in the reported effects. Childs et al. (1981) observed that after exposing Drosophila larvae to pulsed ultrasound, the effects depended strongly on temporal peak intensities and not average intensities. Sikov & Hildebrand (1977), after exposing rats to pulsed ultrasound, observed the "defects showed a better correlation with peak intensity than with average intensity." Few studies have been designed to investigate the relevance of temporal peak versus temporal average intensity in assessing biological effects. Additional information in this area is needed to assess the potential risk of diagnostic pulse-echo ultrasound.

In addition, most of the work so far has involved anatomical end points with functional end points being almost totally ignored. This is another area that should be investigated thoroughly.

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5.4.2 Genetic Effects

Genetic studies on intact animals or humans are difficult to perform because of technical or ethical considerations. Accordingly, studies on the genetic effects of ultrasound have used, in addition to intact animals, such diverse subjects as Drosophila, plants, and cells in culture. In addition to the diverse subjects, a wide variety of exposure parameters has been used, which further complicates the comparison of effects obtained in different studies.

This review will cover the effects of ultrasound on the following: DNA degradation in vitro, chromosome aberrations, mutagenesis, and other indicators of genetic damage. For the purpose of this review genetic effects will include heritable effects or indications of DNA damage in somatic cells as well as genetic cells.

5.4.2.1 DNA Degradation In Vitro

Because the effects of ultrasound on biomolecules are dealt with in section 5.2 of this paper, the in vitro studies of the effect of ultrasound on DNA will only be summarized here. Hill et al. (1969) found that exposure of calf thymus DNA and salmon sperm DNA to 400 mW/cm^2 resulted in DNA degradation. Similarly, Galperin-Lametre et al. (1975) reported that exposing calf thymus DNA to 200 mW/cm^2 ultrasound resulted in DNA degradation. The DNA strand breakage was thought to be due to hydrodynamic shear stress. Combes (1975) exposed transforming DNA in vitro to either 5 or 10 W/cm^2; although the transforming titer of the DNA was sensitive to ultrasound, no increase of mutagenic lesions was seen in the DNA.

5.4.2.2 Chromosome Aberrations

A number of early studies (for review, see Thacker, 1973) found chromosome aberrations in plant root tips. In most studies, the damage was thought to be a result of cavitation or heating. However, Slotova et al. (1967) reported chromosome aberrations in Vicia faba root tips with ultrasound intensities of 200 to 300 mW/cm^2 for 1 to 20 minutes, with the number of aberrations returning to normal levels 24 hours post irradiation. Gregory et al. (1974) and Cataldo et al. (1973), using intensities of 1 to 20 W/cm^2 for up to 2 minutes did not observe any "classical" chromosome aberrations in Vicia faba root tips. They did, however, report the appearance of bridged and agglomerated chromosomes in the exposed cells, and not in the control cells. The authors suggest that the standard chromosome aberration scoring technique would not be suitable for the type of damage seen in these studies, because the "standard" technique is to choose only well-spread metaphase chromosomes for scoring. The significance of the bridged and agglomerated chromosomes is unknown.

In the early 1970's a number of studies were done on chromosome aberrations in human and other mammalian cells after ultrasound irradiation. These studies were stimulated, at least in part, by Macintosh & Davey (1970,1972) who reported the production of chromosome aberrations in human lymphocytes. Other studies (for example, Boyd et al., 1971; Watts et al., 1972; Hill et al., 1972; Buckton & Baker, 1972; and Rott & Soldner, 1973), however, using a variety of parameters (e.g., frequency, intensity, duration of exposure, and cell stage) found no evidence of chromosome aberrations after ultrasound exposure. Two studies (Watts & Stewart, 1972; Galperin-Lamaitre et al., 1973) in which the cells were exposed in vivo also failed to show evidence of the induction of chromosome aberrations. Because of these negative findings, Macintosh et al. (1975) repeated the earlier work (Macintosh & Davey, 1970 and 1972) but were unable to reproduce the positive findings found previously. The preponderance of evidence suggests that diagnostic levels of ultrasound do not cause chromosome aberrations in mammalian cells, although this does not negate the possibility of other genetic damage.

5.4.2.3 Mutagenesis

Wallace et al. (1948) reported the induction of mutations in Drosophila melanogaster after ultrasound exposure. Both lethal and visible mutations were found, although the significance of the mutations is not clear because of the uncertainty of the methodology and intensities used. Kato (1962 a,b) also reported the induction of dominant lethals in Drosophila; once again, the methodology and intensities used are not clear, although it would appear that the intensities were high. On the other hand, Fritz-Niggli & Boni (1950), using intensities of 0.3 to 1.75 W/cm^2, did not find evidence of recessive or dominant lethal mutations; however, the number of subjects tested was small.

Thacker (1974) used the yeast Saccharomyces cerevisiae to test for genetic effects of ultrasound. Two of the assays were tested for mutations in nuclear genes, one for mutation in mitochrondrial DNA, and one for recombination of a nuclear gene. The exposure parameters were similar to those used in diagnostic ultrasound (peak intensity of 10 W/cm^2, using 20 μsec pulses and a duty factor of 0.004) or therapeutic ultrasound (5 W/cm^2 continuous wave for up to 30 minutes). Tests were also made under more severe

conditions than those found in medical applications. The tests showed no evidence of increased mutations or recombination after ultrasound exposure, except under conditions where heat or H_2O_2 was allowed to accumulate.

In another mutation study, Thacker & Baker (1976) tested for evidence of mutation in Drosophila after exposure to diagnostic levels of ultrasound. The system used assayed for recessive lethal mutations using the Muller 5 system, and also for nondisjunction. There was no evidence of mutations or nondisjunction with ultrasound intensities up to 2 W/cm^2, even though these levels were high enough to cause considerable killing of flies.

Bacteria have also been utilized to test for mutation induction after exposure to ultrasound. Combes (1975) used Bacillus subtilis to test for reversion of an auxotrophic mutant after ultrasound exposure. No mutants were seen in this system after exposure to intensities of up to 60 W/cm^2 of pulsed 2 MHz ultrasound. As mentioned earlier, there was no increase in the mutation rate seen after transforming DNA was exposed to ultrasound in vitro.

Lyon & Simpson (1974) tested for genetic damage in mice whose gonads had been exposed to 1 W/cm^2 of continuous wave or pulsed ultrasound at a frequency of 1.5 MHz. They tested for induction of translocations or chromosome fragments in spermatocytes, and for the induction of dominant lethal mutations in females. The tests were negative, but because of sample variation and the number of animals used, only large mutagenic effects would have been observed.

In a recent report, Liebeskind et al. (1979a) found that ultrasound affected several test systems in cultured mammalian cells, suggesting possible genetic damage. A diagnostic ultrasound device was used, and cells were exposed to 2.5 MHz of pulsed ultrasound for 20 to 30 minutes at a spatial peak, temporal peak intensity of 35.4 W/cm^2 (17 mW/cm^2; spatial average temporal average). One test system involved antinucleotide antibodies which are specific for single-stranded or denatured DNA. The cells normally bind these antibodies during the DNA synthesis or S-phase, and have low binding during the G-1 phase. After ultrasound exposure, the cells showed increased binding during the G-1 phase, although there was no evidence of strand breakage as indicated by alkaline-sucrose gradient ultracentrifugation.

Another test system used in this study was the incorporation of ^3H-thymidine into non-S-phase cells as a measure of repair synthesis. Exposure to ultrasound resulted in an increased labeling in the non-S-phase cells, suggesting that repair synthesis was increased. There was, however, no evidence of an increase in sister chromatid exchange which is usually considered a measure of DNA damage.

In the same study, Liebeskind et al. (1979a) also tested for the effects of ultrasound exposure on the morphological transformation of 10T-1/2 cells. It was found that ultrasound resulted in the induction of Type II morphological transformants, both with and without the promoter TPA.

In a subsequent study, Liebeskind et al. (1979b) showed that diagnostic levels of ultrasound induced small, but significant, increases in sister chromatid exchange in fresh human lymphocytes as well as in a human lymphoblast line. Similar results have been obtained by Haupt et al. (1981). The significance of sister chromatid exchange is unknown, but it does appear to reflect chromosome damage. The increased sister chromatid exchanges reported in these papers are contrary to the results seen by Morris et al. (1978). Human leucocytes were exposed to 15.3 to 36 W/cm^2 for 10 minutes to cw ultrasound at a frequency of 1 MHz. No increase in sister chromatid exchanges was observed after exposure. The reason for the different results in these two studies is unknown, but one possibility is the difference in exposure regimens.

It is not clear whether the Liebeskind (1979a, 1979b) reports indicate genetic damage. The immunoreactivity seen suggests disturbances in cellular DNA, but other interpretations

are possible. The density gradient analysis does not appear to indicate DNA strand breakage. The transformation data suggest possible genetic damage, since genetic damage appears to play a role in cellular transformation. Three types of abnormal morphology of transformed cells have been described (Reznikoff et al., 1973). The cells used in this study had Type 1 morphology to begin with and the ultrasound treatment transformed a few of the colonies to Type II morphology. Because transformation does not appear to to be a sudden event, but rather a progression of changes (or stages), and because the transformation seen in this study is apparently only a part of that progression, it does not necessarily follow that genetic damage has occurred. It is significant, however, that ultrasound had an effect on the process of transformation.

At present, it is not certain whether ultrasound affects genetic material. It may be that high intensities can cause genetic damage (early Drosophila work) since under certain conditions, ultrasound can destroy tissue. However, for exposure conditions resembling those used diagnostically or therapeutically, the issue is anything but certain. Isolated DNA can be degraded with relatively low levels of ultrasound, but the same degradation may not occur in vivo, at least not at low intensity exposure.

At this time there seems to be little evidence that ultrasound produces mutations or chromosome aberrations in mammalian cells. The best evidence of a possible genetic effect is presented by the transformation and sister chromatid exchange data, which do not by themselves prove genetic damage, but do strongly suggest it. Further mutation and transformation studies with mammalian cells are needed to assess the potential for genetic damage from ultrasound exposure. The possible role of cavitation in producing effects in cell suspension systems and the relevance of cavitation under in vivo conditions must also be considered.

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5.4.3 Immunologic Effects

The increasing use of ultrasound for both diagnostic and therapeutic purposes has stimulated research in the biological effects of ultrasound radiation. The effects of ultrasound on the immune responses have not been extensively investigated, although there are a few recent reports of immune effects from ultrasound exposure.

Anderson & Barrett (1979) reported a dose-dependent immunosuppressive effect in mice exposed to pulsed ultrasound. Mice were given a 5-minute exposure to 2 MHz ultrasound at a maximum spatial average, temporal average intensity of 8.9 mW/cm^2 (spatial peak, temporal peak of 28 W/cm^2) applied over the area of the spleen. After an injection of sheep erythrocytes (SRBC) the hemagglutinin and hemolysin antibody titers were slightly reduced in the mice receiving ultrasound treatment. Although the results suggest that the antibody response to SRBC was diminished after ultrasound, the complexity of this response, as well as the assay techniques used, warrants cautious interpretation of this data. In an attempt to confirm these results, Child et al. (1981) were unable to show a reduced hemagglutinin or hemolysin response in sera from mice sonicated earlier. These negative results were obtained with conditions similar to those used by Anderson & Barrett (1979), and with intensities tenfold higher. The reason for these differences is not known.

Anderson & Barrett (1981) reported further immunological effects of ultrasound. Exposure of the liver area in mice to ultrasound (2 MHz, 8.9 mW/cm^2, 691 pulses/sec) caused a depression of phagocytosis as measured by carbon clearance. This effect was evident at 48 and 72 hours after exposure. It was also found that there was a decrease in the ability of peritoneal macrophages from treated animals to phagocytose or kill bacteria as compared to macrophages from untreated animals.

Recently, Liebeskind et al. (1979) used immunological techniques to assess the bioeffects of diagnostic ultrasound. It was reported that specific antinucleotide antibodies which demonstrate specificity to single-stranded or denatured DNA detect changes after ultrasound exposure. This increased immunoreactivity was studied in vitro using synchronized HeLa cells exposed to ultrasound. Thus, it appears that immunological techniques may be useful in assessing deleterious effects of ultrasound.

In another study the exposure of tumor cells to ultrasound or ultrasound plus x ray reduced the electrophoretic mobility of the cells by 30 percent (Repacholi, 1970). The author hypothesizes that ultrasound may have been capable of shearing the mucopolysaccharide coat from the tumor cell, thus enhancing the potential of lymphocytes for tumor cell killing.

Many experiments were performed and interpreted prior to the development of concepts that underlie current thinking with respect to cellular immunology, including the definition of subpopulations of lymphocytes and their known cooperation in the immune response. Since it is now possible to dissect many individual components of the immune response and to isolate functionally uniform subpopulations of lymphocytes, it is conceivable to design experiments which will delineate the effect of ultrasound on various immune phenomena. For example, since ultrasound does not appear to destroy immunogenicity of tumors, but rather enhance it, further studies are warranted on the possible cumulative effects of combined immunotherapy and radiotherapy (ultrasound and x ray) on certain tumors. Further studies are clearly required with due consideration to radiobiological parameters such as dose, dose rate, and quality of irradiation, as well as persistence of the effect.

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5.4.4 Hematologic/Vascular Effects

A growing body of experimental evidence suggests that the cellular elements of blood are adversely affected by exposure to ultrasound. Diagnostic intensities of ultrasound may cause shifts in the hemoglobin dissociation curve and the loss of erythrocyte blood group surface antigens, while therapeutic intensities have been implicated in the lysis of erythrocytes, leukocytes, and platelets, as well as in alterations in the metabolic activities of leukocytes. The sensitivity of cells in a fluid medium to the shear stress forces produced by ultrasonic cavitation has been implicated as the mechanism for the interaction of ultrasound with blood cells.

5.4.4.1 Platelets

Blood platelets, the cellular elements of blood responsible for the initiation of clotting, are highly sensitive to shear stress. Stresses as low as 50 dynes/cm^2 produced in vitro by a rotational viscometer cause platelets to swell, while higher stresses induce aggregation and fragmentation (Brown et al., 1975). Williams (1974) demonstrated serotonin release from platelets subjected to shear stresses as low as 130 dynes/cm^2. A small portion of the platelet population is particularly sensitive to shear stress and can be ruptured at levels that might only induce swelling of other platelets (Hung et al., 1976). The existence of such a subpopulation is of importance because intracellular substances released from ruptured platelets can activate other platelets to release aggregating (clot promoting) agents with the resulting formation of small emboli. Shear stress also has an adverse effect on the structure and mechanical integrity of experimentally induced clots, reducing the mechanical strength of a clot to as little as one-fifth of normal, with a shorter formation time (Glover et al., 1977).

The shear stress forces associated with ultrasonically induced cavitation have been implicated in a variety of platelet effects (Sykes & Williams, 1977). Ultrasound exposure at a frequency of 1 MHz lowers the recalcification time of platelet-rich plasma at intensities as low as 0.065 W/cm^2 (spatial average) (Williams et al., 1976a). Ultrasound was proposed to increase the availability of platelet factor 3, thus accelerating the rate of fibrin formation (Williams et al., 1975). Subsequent morphological analysis of recalcified clots revealed the presence of platelet debris (Williams et al., 1976b). The ultrasound had apparently ruptured a small portion of the platelet population, releasing adenosine diphosphate (ADP) and other aggregating agents into the surrounding plasma. These agents then induced other platelets to release, resulting in a self-perpetuating cycle of platelet aggregation and release. As with shear stress, the clots were less dense and rigid than normal clots.

Other in vitro studies have demonstrated direct platelet aggregation upon exposure to ultrasound (Chater & Williams, 1977). Loss of platelet shape was noted at an intensity of 2 W/cm^2 (spatial average) and a frequency of 0.75 MHz, with a progressive increase in aggregation above 3 W/cm^2. Electron microscopic examination revealed damaged platelets in all exposed samples. The threshold for the effect varied with the individual sample, and was directly related to the ADP sensitivity of the sample. Thus, subjects with high ADP sensitivity were more sensitive to ultrasonically induced aggregation. At subthreshold intensity, exposure produced a refractory state in platelets, making them less sensitive than normal to ADP. Platelet aggregation at still lower intensities has been found in an experimental system known to contain gas bubbles of resonant size (Miller et al., 1978, 1979). In this system, cavitation induced platelet aggregation at levels as low as 0.032 W/cm^2 (spatial peak) of 2.1 MHz continuous wave ultrasound. It was further noted that the bulk of the aggregation occurred early in the exposure period. In an important comparison, pulsed ultrasound with 1 ms bursts and a 10 percent duty cycle doubled the threshold to 0.064 W/cm^2 (temporal peak; 0.0064 W/cm^2 temporal average). The Miller et al. (1979) study is the sole in vitro comparison of the effects of pulsed versus continuous wave ultrasound on blood cells.

Little information exists in the literature on the effects of ultrasound on platelets in vivo. Williams (1975, 1977) demonstrated that shear stress forces similar to those that might be generated in vivo by acoustic cavitation can cause significant changes in blood flow when produced intravascularly in mice. Effects ranged from platelet adhesion to the endothelial walls of the blood vessel to clot formation and complete occlusion of the vessel. Microscopic examination revealed that platelets had been ruptured by the stress forces, releasing clot promoting substances into the surrounding plasma. The degree of the effect might then be considered dependent on the number of sheared platelets and the velocity of blood flow.

Shear stress forces of sufficient magnitude to disrupt platelets can apparently be generated in vivo by ultrasound. Zarod & Williams (1977) found small platelet aggregates within the microcirculation of the guinea-pig pinna after exposure to ultrasound at either 0.75 or 3.0 MHz for 2 minutes at 1 W/cm^2 (spatial average).

The actual formation of clots or microemboli is not, however, the only potential in vivo effect of ultrasound exposure. Platelets respond in varying degrees to external stimuli. As noted by Chater & Williams (1977), platelets only partially stimulated by ultrasound are less likely to respond to other stimuli, such as ADP, for a period of time. Such an effect has been demonstrated in vivo by Lunan et al. (1978), who found decreased aggregation of platelets after exposure of mice to 2 MHz ultrasound at 1 W/cm^2 (spatial average).

The effects of ultrasound on human platelets irradiated in vivo are only beginning to be investigated. Williams et al. (1977) measured beta-thromboglobulin release by human platelets after in vivo exposure to ultrasound at 0.5 W/cm^2 (spatial average) at a frequency of 0.785 MHz and found no detectable changes above controls values. However, subsequent in vitro experiments indicated that the 0.5 W/cm^2 intensity may have been subthreshold. Samples of platelet-rich plasma released beta-thromboglobulin when exposed to ultrasonic intensities above 0.6 W/cm^2 (Williams et al., 1978). However, Sanada et al. (1977) noted reversible changes in platelet shape and retention ratio in human subjects given a diagnostic cardiac exposure of 0.005 W/cm^2 (spatial average) at 2.25 MHz for up to 40 minutes. Since platelet shape changes are an early indication of platelet stimulation, these results indicate the potential of a clinical effect at higher intensities.

Dyson et al. (1971, 1974) exposed live chick embryos to 3 MHz ultrasound and found blood stasis (i.e. stopping the blood flow) occuring at levels as low as 0.5 W/cm^2 (spatial average). The threshold for an individual vessel varied with its type, size, and orientation and also with the embryo's heart rate. Large-bore vessels had lower thresholds than small-bore vessels, and veins were more sensitive than arteries of similar diameter. Lower thresholds also accompanied lower heart rates. Complete stasis was favored in vessels oriented parallel to the direction of sound propagation. The effect could be eliminated

completely if the transducer was moved during exposure. The mechanism of action is unclear, but appears to involve the presence of standing waves produced by tissue reflections (Baker, 1972).

When the transducer is moved during exposure, ultrasound can actually increase blood flow (Paul & Imig, 1955). This effect is related to the tissue hyperthermia associated with long exposure times and relatively high intensities.

Ultrasound-induced platelet effects could have serious clinical consequences. The production of platelet aggregates in vivo might, for example, lead to the blockage of circulation in small capillaries and subsequent complications of embolism and infarction, especially in patients exhibiting clinical conditions which might predispose them to thrombosis (e.g., pregnancy, post surgery). Premature removal of platelets damaged by ultrasound might exceed a patient's capacity for replacement and give rise to clinical complications associated with thrombopenia. The nature and extent of possible complications from ultrasound-platelet interactions have yet to be fully evaluated. Some of these interactions may, in fact, be beneficial. For example, Hustler et al. (1978) found inhibition of experimental bruising of guinea pig ear after exposure to 0.6 W/cm^2 at 0.75 MHz.

5.4.4.2 White Cells

A study by Crowell et al. (1977) is the only clue to the possible effects of ultrasound on white blood cells. Their shear stress resistance was tested and found to be more than 3 times higher than that reported for platelets. The stress thresholds for functional changes as measured by reductions in phagocytic ability, bactericidal capacity, and oxygen consumption were, however, lower than those reported for platelet changes. Ultrasound might well be expected to have an effect on these end points, but this has yet to be tested.

5.4.4.3 Red Cells

Like platelets and white cells, red blood cells are sensitive to rupture by shear stress (Leverett et al., 1972; Nevaril et al., 1968; Rooney, 1970; Williams et al., 1970; Veress & Vineze, 1976/77). While the threshold stress value is more than an order of magnitude higher than for platelets, ultrasound at megahertz frequencies can disrupt red cells in vitro at low intensities. Veress & Vineze (1976/77) found hemolysis occurring at intensities as low as 0.2 W/cm^2 (spatial average). It was not determined whether this represented a threshold value, but a linear relationship existed between the logarithm of the time necessary to produce hemolysis at 1 MHz and the intensity of the ultrasound at a given concentration of blood cells. A significant hemolysis of red blood cells exposed to diagnostic Doppler units with intensities estimated in the range of 10 to 20 mW/cm^2 after 6 to 8 hours exposure was reported by Hikaru & Noriyuki (1977). Hemolysis of samples exposed to a diagnostic pulse system was reported higher than for the control group but much lower than for samples exposed to a Doppler diagnostic unit.

Nonlytic effects of ultrasound on red cells in vitro have been reported by Bundy et al. (1978), who observed a reduction in nonmediated leucine transport across the erythrocyte membrane following a 30-minute exposure at 0.6 W/cm^2 (spatial average; 1 MHz), and Pinamonti et al. (1977) who noted a shift in the hemoglobin dissociation curve and a loss of erythrocyte surface antigens at .008 W/cm^2 (8 MHz). Lota & Darling (1955) reported an increased permeability of the human erythrocyte for K^+ions at 37 °C with continuous wave ultrasonic exposures of 0.5 to 3 W/cm^2 for 5 and 30 minutes. The amount of loss of K^+ions from the erythrocyte increased with both intensity and exposure time. However, in the absence of other studies, it is impossible to predict what functional effects ultrasound might have on red cells in vivo.

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5.4.5 Neurologic, Behavioral, and Sensory Effects

5.4.5.1 Neurologic and Behavioral Effects

Perhaps the best-documented bioeffect of ultrasound radiation is the production of lesions in the central nervous system (brain and spinal cord) of experimental animals.

Starting in the early 1950's, there were reports of spinal paralysis produced using current therapy devices (Anderson, 1951). Subsequently, a decade of intensive effort was directed toward development of a method for the production of small, precisely placed deep brain lesions without producing injury to the intervening brain tissue (Fry, 1953, 1958; Wall et al., 1953).

To produce such lesions, investigators removed the bone overlying the neural tissue and applied ultrasound directly to the brain or spinal cord at intensities ranging from about 40 W/cm^2 (Basauri & Lele, 1962) to about 1000 W/cm^2 (Fry et al., 1954). Because of the surgical procedures and the high intensities used, these studies have no direct applicability to the evaluation of the safety of current diagnostic equipment. However, this research can provide interesting leads for future research and caveats for ultrasound use. An example is the research by Taylor (1970) and Taylor & Pond (1972), who exposed the spinal cords of rats to pulsed ultrasound at frequencies of 0.5 to 6 MHz, with peak intensities of either 25 or 50 W/cm^2. By varying the pulse duty cycle and the total exposure duration, they were able to produce spinal cord damage at time average intensities as low as 2.5 W/cm^2. Within the range studied, damage was inversely related to the ultrasound frequency, with 5 MHz producing no damage. Additionally, hypoxia rendered the tissue more susceptible to damage by 40 percent. More recently, Stolzenberg and his associates (1980) produced spinal cord damage in intact animals by sonicating for 120 s at 1 W/cm^2 at a frequency of 2 MHz, confirming, using more sophisticated equipment and dosimetry, the findings of Anderson (1951). Similarly, Borrelli (1981) has provided information elucidating the early changes preceding the frank tissue destruction seen by Fry and other early workers. Borrelli reports that morphology of the chemical synapse is altered by exposure of the exposed cat brain at an SPTP intensity of 30 W/cm^2.

A few studies show that ultrasound can produce reversible effects on both central and peripheral neurons. Exposure can produce alterations of nerve function as measured by conduction velocity (Madsen & Gersten, 1961; Esmat, 1975), action potential (Shealy & Henneman, 1962; Young & Henneman, 1960; Takagi et al. 1960) or electroencephalograph (Hu & Ulrich, 1976; Kohorn et al., 1967). The results of these studies are quite contradictory, with electrophysiologic measures showing both increases and decreases. Because of differences in ultrasound exposure parameters, species differences, and dosimetric uncertainties, the only conclusion that can be reached from these studies is that continuous wave power densities as low as 0.5 W/cm^2 can produce transient alterations in neural function. The possible health significance of these findings cannot be evaluated until more neurological data are obtained to resolve the apparent contradictions in enhanced versus suppressed neural function.

Because of the extensive use of ultrasound in obstetrics, the effects of ultrasound on fetal tissue have begun to receive extensive research interest. The teratological research is reviewed elsewhere in this document. Of particular interest here are the potential behavioral sequelae of in utero exposures. Murai and coworkers (1975a; 1975b) reported two studies on functional effects of the cw exposure of fetal rats to ultrasound. Physically restrained dams were exposed to 2.3 MHz ultrasound at an intensity of .02 W/cm^2 for 5 hours on the 9th day of gestation, with tests for normal behavioral development of offspring immediately postpartum (1975a) or at 120 days of age (1975b). In the first study, Murai et al. reported a significant delay in maturation of the grasp reflex in exposed offspring relative to sham exposed and untreated controls. They also found significant differences in both exposed and sham exposed groups relative to untreated controls in vibrissa placing response, visual placing response, and acceleration righting reflexes. The similar effects on sham and exposed subjects were attributed to the maternal stress of immobilization during exposure. The authors suggest, however, that the possibility of prenatal ultrasound effects per se cannot be excluded as a contributing factor. Murai et al. (1975b) used similar exposure procedures, but tested for emotional and cognitive behaviors in 120-day-old rats. Vocalization to handling and escape responses from electric foot shock (emotional behaviors) were significantly increased in exposed versus sham and untreated controls. Discrimination learning of horizontal versus vertical strips (cognitive learning) was equal and unaffected in all groups. Murai et al. concluded that the emotional behavior of rats

can be influenced by prenatal exposure to ultrasound intensities as low as .02 W/cm^2, and suggested the need for further studies of latent and functional postnatal effects of even low-level fetal exposure.

The altered postnatal behavioral changes reported by Murai et al. have now been replicated by Sikov et al. (1976). Rat dams were exposed to 0.93 MHz continuous wave ultrasound for 5 minutes on the 15th day of gestation, with average intensities of 0.01, 0.04, 0.17, 0.54, or 1.0 W/cm^2. Again, the grasp reflex was affected in all ultrasound intensity groups, with only 50 to 80 percent of subjects in each intensity group displaying grasp reflex by day 6. Similar behavioral deficits were reported for the righting reflex, head lift, and holding responses. Sikov et al. concluded that the threshold for postnatal effects must be less than 10 mW/cm^2. Additionally, the authors observe that the deficits were not permanent, but rather were transient delays in maturation relative to normal controls.

Summary to Section 5.4.5.1

Postnatal functional data, if confirmed, present a serious challenge to the assumption that fetal exposure to ultrasound is innocuous and also raises the possibility that functional/neurological thresholds might be more useful in setting exposure limits than thresholds based on prenatal assessment of anatomical and morphological end points.

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5.4.5.2 Effects on the Eye

The application of pulsed and cw ultrasound is now a widely accepted diagnostic technique to image the retina, lens, and other internal structures of the eye, especially in patients with opaque or semi-opaque corneas and lens. Using B-scan imaging systems, physicians can diagnose ocular abnormalities, such as retinal detachments, tumors, macular lesions, vitreous hemorrhage, and intraocular foreign bodies in cases where conventional ophthalmoscopy is not possible (Lizzi et al., 1976; Baum & Greenwood, 1960; Sutherland & Forrester, 1974). Therapeutic applications of ultrasound include disintegration of the lens in cataract surgery (Kelman, 1967; Emery, 1974a)--a technique known as phacoemulsification--and treatment of certain types of glaucoma (Lizzi et al., 1978), myopia (Greguss & Bertenyi, 1976), and detached retinas (Lizzi et al., 1978a; Purnell et al., 1964).

Phacoemulsification is a surgical technique that involves dissolving, emulsifying, and aspirating a mature cataract utilizing kHz ultrasound (Kelman, 1967; Emery, 1974a). Properly performed, the procedure gives final visual results essentially the same as intracapsular extraction (Emery et al., 1974b), with post-operative vision better than preoperative vision in 98.4 percent of patients treated (Emery & Paton, 1974c). Controversy has arisen, however, over the potential adverse effects of ultrasound from phacoemulsification. Thermal effects to the cornea and iris can occur whenever the flow of the irrigating solution is inadvertantly shut off. The irrigating solution normally lowers the temperature of the anterior chamber 4 to 5 °C. With the ultrasonic probe running, the temperature increases 4 to 5 °C to a normal level. If irrigation ceases, within 1 minute, the temperature can rise enough to denature protein (Emery, 1978). Although neither Emery (1974b) nor Kelman (1967) detected corneal endothelial effects arising from the use of ultrasound in phacoemulsification, Ito (1970), Binder et al. (1976), Polack & Sugar (1976), and Olson et al. (1978 a&b) have found widespread damage in cats and rabbits, particularly in cases where the ultrasonic probe was activated inside the anterior chamber. The

damage, however, was generally reversible within 24 hours, resulting in agreement with Kelman (1973), who noted that in actual clinical practice corneal complications, however induced, last only a few days. Nontheless, considering the limited repair capability of the adult corneal endothelium (Emery, 1978), the period of ultrasound exposure during phacoemulsification should be kept as short as possible.

As far as diagnostic applications are concerned, Baum (1956) considers 0.25 W/cm^2 (1 MHz) for 5 minutes the upper limit of safety. Reversible effects, including vascular engorgement of the conjunctiva and transient corneal opacities, were detected in the range of 1.5 to 2.0 W/cm^2 for 5 minutes with irreversible effects occurring above 2.5 to 3.0 W/cm^2 for 5 minutes. Jankowiak et al. (1964), however, reported superficial erosions of the cornea, slow pupillary reactions to light, indistinct structure of the ocular fundus, and erythrocytic extravasation in the eyes of rabbits exposed to 1 MHz ultrasound at either 0.05 W/cm^2 for 1.5 minutes, 0.1 W/cm^2 for 1.0 minute, or 0.2 W/cm^2 for 0.5 minute. Preisova et al. (1965) measured significant changes in corneal temperature at intensities above 0.5 W/cm^2 7 MHz. Transient decreases in intraocular pressure have been reported following repeated 10-minute exposures to 1.5 W/cm^2 at 0.8 MHz (Bernat et al., 1966) and repeated 2 to 45 second exposures to 12 W/cm^2 at 3.0 MHz and 0.1 W/cm^2 at 7.0 MHz (Rosenberg & Purnell, 1967). Cumulative effects of repeated low-level ultrasound at 1 MHz and 0.1 W/cm^2 have been reported to result in demyelinization of the optic nerve, atrophy of the retina, and eventually lesions in the visual centers of the brain (Jankowiak & Majewski, 1965). In another study of effects at low levels, Ziskin et al. (1974) failed to detect any morphological changes in the eye exposed to 9.4 MHz ultrasound at 3.37 mW/cm^2 for up to 4 hours. Barnett & Kossoff (1977) similarly failed to detect changes in the lens and retinas of cats exposed to 7.5 MHz pulsed ultrasound at 1.3 mW/cm^2 (temporal average) for 30 minutes.

The lens is often cited as being the most susceptible part of the eye to ultrasound, but in fact the cataractogenic dose appears to be quite high. Gavrilov et al. (1974) noted evidence of cataract formation in rabbits after exposure to 2.7 MHz at intensities of 400 W/cm^2 and above. Moiseeva & Gavrilov (1977), using 2.09 MHz ultrasound reported lens opacities at intensities of 250 to 600 W/cm^2. Lizzi et al. (1978b) reported cataract production in rabbits using 9.8 MHz ultrasound at intensities above approximately 200 W/cm^2. The damage threshold described by Lizzi et al. (1978b) was reported to be 10^4 times higher than current diagnostic instruments.

Lower thresholds in the ranges of 30 to 150 W/cm^2 were reported by Sokullu (1972) for 1 to 7 second exposures to a pulsed source at 3.5 MHz. Additionally, Sokullu notes that at lower power levels the cataractogenic effect of ultrasound can be eliminated if the ratio of pulse length to pulse interval is less than 1:1. For high energy pulses, the pulse interval may be increased to 5 times the pulse length without significant effect on cataract production. Repeated exposures, however, appear to lower cataract threshold significantly. Bernat et al. (1966) report cataract production in rabbits following an exposure regimen of 10-minute exposures to 1.5 W/cm^2 at 0.8 MHz 3 times a day for 12 to 14 days. In the opinion of some investigators, ultrasound induced cataracts appear to arise from a thermal mechanism (Lerner et al., 1973) regardless of the time-intensity parameters of ultrasound induced cataracts from a thermal mechanism (Lerner et al., 1973).

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5.4.6 Skeletal, Organ and Soft Tissue Effects

A number of skeletal and soft tissue effects have been reported following exposure to ultrasound. In this section they are divided into effects on the skeletal system, muscles, other organs and tissues, tissue regeneration, and neoplastic tissue.

5.4.6.1 Effects on Bone and Muscle

The practice of ultrasound diathermy commonly involves deliberate exposure of muscular and skeletal tissue. One such use is the treatment of contractures attributable to scarring and other disorders related to the limited extensibility of collagenous tissue (Lehmann et al., 1974). The clinical literature (e.g., Lehmann & Guy, 1972; Gersten, 1955) and some laboratory research (Bierman, 1954) confirms the positive effects of ultrasound exposure on the extensibility of fibrous tissue. However, adequate comparisons of the efficacy of ultrasound and other modes of heating have not been made. More recent findings suggest that ultrasound therapy may lessen collagen formation and scarring in coronary infarcts (Franklin et al., 1977).

Skeletal tissue may be exposed to ultrasound for therapeutic purposes or incidentally in therapy and diagnosis. Goldblatt (1969) exposed rabbits with a fracture of the third metatarsal to ultrasound intensities of 0.4 W/cm^2 or 0.8 W/cm^2. As many as 15 treatments were given. A statistically significant enhancement of healing was observed during treatment, but after 45 days post-treatment, no differences were observed between experimental and control groups.

On the other hand, ultrasound exposure can result in significant adverse effects on bone. In an early study, Barth & Wachsmann (1949) reported grossly observable losses of

periosteum and a cessation of marrow growth following exposures of about 3 W/cm^2, average intensity. Kolar et al. (1964) reported long-lived depression of calcium metabolism in bone treated at intensities of about 4.5 W/cm^2. Such changes could result in altered bone growth. Both Payton et al. (1975), using repeated exposures of 2.5 W/cm^2 exposures, and Bender et al. (1954), using single 500 mW/cm^2 exposures, reported striking deterioration of bone marrow and alterations in periosteum of dog femurs. Payton et al. (1975), also reported alteration of the femoral cortex.

Alteration of smooth muscle contractability has been reported in a number of studies. Both Talbert (1975) and ter-Haar et al. (1978) were able to provoke increased uterine contractions by sonication at intensities of 3 W/cm^2 or 2 W/cm^2, respectively. In a related study, Hu et al. (1978) studied the effects of low-level ultrasound on rat intestinal muscle. They reported that 1 MHz ultrasound at intensities of 1.5 W/cm^2 for 5 minutes inhibited action potentials of the smooth muscle. For single exposures, this effect was observed to be reversible and recovery complete. However, when repeated ultrasound exposure was given, only a partial recovery was observed and the effects seemed to be cumulative. The presence of high calcium ion concentration in the medium prevented the observed effects suggesting that ultrasound may have acted on the calcium influx mechanism of the cell membrane. However, the study by Talbert (1975) showed no interaction with increased calcium concentration.

Mortimer and his associates have investigated the effect of ultrasound on a variety of cardiac muscle functions. Using cardiac muscle preparations (Mortimer et al., 1978; 1981) and isolated heart preparations (Mortimer et al., 1980), these workers have demonstrated changes in cardiac mechanics at SATA intensities as low as 0.7 W/cm^2. Results of such studies provide useful leads for in vivo research.

It has been generally assumed since the inception of ultrasound diathermy that sonication increased blood flow to skeletal muscle (see, for example, Buchan, 1970). Experimental results have been reported showing such increases (Abramson et al., 1960) in perfusion as a result of sonic exposure. However, Hogan et al. (1981) have recently shown that profusion can be increased in ischemic muscle and decreased in non-ischemic muscle sonicated at the same time. Since the effects of ultrasound on perfusion of skeletal muscles apparently depend on unknown physiological factors, caution must be used in ascribing beneficial effects via increased blood flow.

5.4.6.2 Effects on Organs

Hrazdira and Konecny (1966) reported a suppression of radioiodine uptake in rabbits after repeated 5-minute exposures of the thyroid region to therapeutic levels of ultrasound (1-2 W/cm^2, 0.8 MHz, continuous wave). Based on histological evidence, the authors suggested the effect may have been the result of an impaired ability of the follicles to concentrate inorganic iodine.

Kremkau and Witcofski (1974) reported a reduction in the mitotic index of regenerating rat liver tissue, measured 30 hours after 5 minutes exposure to 60 mW/cm^2, 1.9 MHz, continuous wave ultrasound. However, another group of investigators (Miller et al., 1976) observed no effect, under similar experimental conditions, after exposure of up to 16 W/cm^2, 2.2 MHz, continuous wave ultrasound. One possible significant difference between the two studies is that the transducer was held stationary during exposure in the former study and moved in a circular motion over the region of the liver in the latter study. Barnett and Kossoff (1976) exposed regenerating rat liver to pulsed ultrasound (33 W/cm^2, peak instantaneous intensity, 50 kHz pulse repetition, 30 minutes exposure) and observed no decrease in mitotic activity or significant difference in the relative amount of regenerated tissue.

Stephens et al. (1978) observed ultrastructural changes in the mitochondria of several tissues, including the liver, pancreas, kidney and adrenal from mice exposed to spatial average intensities as low as 1 W/cm^2, 2 MHz, continuous wave ultrasound, for 500

seconds. The investigators indicated that the mitochondrion appeared to be the primary subcellular organelle affected by ultrasound exposure and the mitochondrial abnormalities appeared to be irreversible.

Fahim et al. (1975) compared the effectiveness of ultrasound exposure for inducing sterility in male rats (1.0 W/cm^2 for 5 minutes; 1.0 W/cm^2 for two 5-minute treatments, separated by 48 hours; or 2 W/cm^2 for 5 minutes) with treatment in a 60 °C water bath, infrared exposure, or microwave exposure. They report that ultrasound was more effective, at lower intratesticular temperatures, than the other treatments. The investigators later reported reduced spermatogenesis in cats, dogs, monkeys and four human volunteers under similar intensities and times (Fahim, et al., 1977). Although the authors suggest that ultrasound treatment may be a means of inducing reversible or irreversible sterility without surgery or drugs (Fahim et al., 1975), the genetic implications of such treatment are not sufficiently understood to endorse its use for human contraception.

5.4.6.3 Effects on Tissue Regeneration

Dyson et al. (1968) observed an increased rate of tissue regeneration after treating experimentally produced surgical defects in rabbit ears with 3.5 MHz ultrasound for 5-minute periods, 3 times a week. Although significant increases in the growth rate of regenerating tissue was observed at intensities as low as 100 mW/cm^2 (continuous wave), the optimum growth stimulating activity was observed at 0.5 W/cm^2 (pulsed 2 msec on, 8 msec off). Slight increases in the temperature of the tissue occurred during exposures. However, the investigators considered the possibility of tissue heating to be an unlikely cause of the increased growth rate. The same investigators (Dyson et al., 1970) also reported an increased rate of ^3H-thymidine uptake by the exposed tissue, as well as histological changes. The structural changes, observed by electron microscopy, were considered as possible evidence that ultrasound may affect protein synthesis and polymerization. The observations of increased tissue regeneration after ultrasound exposure were later extended to the treatment of varicose ulcers in humans. Dyson et al. (1976) reported that the application of ultrasound (1.0 W/cm^2; pulsed 2 msec on, 8 msec off; 3 MHz; up to 10 minutes exposure, 3 times a week) significantly stimulated the healing of chronic varicose ulcers.

5.4.6.4 Effects on Neoplastic Tissues

A considerable amount of research has been done on the treatment of neoplastic tissues with ultrasound either by itself or in combination with radiation or chemical therapy (for review, see Kremkau, 1979). For example, Longo et al. (1975,1976) studied the local and systemic effects of ultrasound on neoplastic tissue. The authors demonstrated that ultrasound inhibited the growth of a transplantable Wilm's tumor in rats, resulting in an increased mean survival time (MST). The MST for control rats was 19 days while the MST was 25 days for the exposed animals. In this experiment, the tumors were exposed on day 10 and day 12 after implantation. The authors also reported that ultrasound exposure resulted in greater and longer lasting growth inhibition of the tumor than previously observed from either whole-body or local irradiation with 600 R x rays. The systemic effects of ultrasound were investigated by implanting a Wilm's tumor on both sides of the rats' bodies. The tumor on the right side was exposed on day 7 after transplantation; a declining growth rate was seen immediately in this tumor, while there was no change in the control (left) side. The tumor weights on day 8 after exposure were 10.3 ± 2.7 g for the exposed tumor and 24.5 ± 4.5 g for the control. There was no increase in the MST since the rats succumbed to the progressing tumor. Thus, it appeared that there was a local anti-tumor with no systemic immunity.

Kishi et al. (1975) studied the effects of focused ultrasound (944 kHz) on the growth of gliomas implanted experimentally into the abdominal walls and brains of mice. They found that intensity levels of 100 W/cm^2 for 10 sec increased the tumor size in the abdomen and decreased slightly but not significantly the survival time. Much higher intensity levels of

ultrasound (1000 W/cm^2 for 1 to 2 sec) reduced the tumor size and increased the survival time significantly when the implants were made in the abdomen or head.

In some cases, ultrasound treatment has been found to enhance the gamma irradiation effect on tumors. For example, Gavrilov et al. (1975) reported that relatively low levels of ultrasound at 880 KHz enhanced the sensitivity of transplanted sarcoma tumors in mice to ionizing radiation. In contrast, Clarke et al. (1970) assessed the possible synergistic effects of ultrasound and x ray at the cellular level with the use of L5178Y mouse lymphoma cells in suspension. In these experiments ultrasound had no significant effect on the survival of the tumor cells either alone or by altering the response to x ray.

The mechanism of how ultrasound affects tumor tissues by itself or in conjunction with other treatments would appear to be that of heating. It is possible that ultrasound could affect tumors indirectly also, by affecting other tissues or systems, such as the immune system, but very little is known about such indirect effects.

Summary for Section 5.4.6

Administration of ultrasound at intensities of 1 W/cm^2 or more can produce increased temperatures in muscle and skeletal tissue. Although a therapeutic benefit may be induced, the therapeutic ratio seems to be very small, since identical exposures can produce profound irreversible damage in bone. Moreover, much research remains to be done before the effect of localized hyperthermia on blood flow can be predicted with confidence.

Most of the effects on organs have been reported from exposure to therapeutic intensities of ultrasound, in some instances involving significant heating of the tissues being studied. Studies of the effect of exposure to intensities of ultrasound in the diagnostic range on mitotic activity have yielded conflicting results. However, there is experimental evidence that ultrasound stimulates wound healing at levels of exposure that do not involve significant heating.

There is evidence that ultrasound can affect tumor tissue, both in vivo and in vitro. Additionally, ultrasound appears to sensitize some tumors to radiotherapy, probably by heating the tissue. Much work needs to be done to optimize these apparent antitumor effects.

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5.5 HUMAN EFFECTS

The medical applications of ultrasound increase in number and scope as this technology becomes more widely available and as technical improvements permit. Currently, an almost bewildering variety of uses exists. For example, ultrasound is used to diagnose pregnancy problems, monitor fetal hearts during delivery, and to evaluate intracranial hemorrhage in neonates. Other applications vary from the diagnosis of cardiac and vascular problems to the measurement of blood flow in a variety of circumstances to the differential diagnosis of childhood abdominal tumors. Current experimental applications include the destruction of tumor tissue (Mallard et al., 1979), and the use of "enhancers" analogous to computer assisted tomography in x-ray technology (Skolnick, 1978; Greenleaf et al., 1978).

The rapid rate at which ultrasound devices evolve is an inextricable element in the evaluation of ultrasound safety. For example, for many years the cost and complexity of B-scan equipment confined it to medical centers. Recent marketing of so-called real-time devices at relatively low cost enables their use in physicians' offices, and now it is possible that large numbers of fetuses are exposed for indications and to exposure characteristics which may be quite different from those which apply to B-scan devices. Further, it is possible that in the future, frequencies will be higher.

In general, assessment of the effect of ultrasound in humans is complicated by the rapid development of ultrasound technology in medicine. Assessment of safety for a given use of a given device would require many years, particularly when one is looking for long-term effects. A comprehensive assessment of ultrasound safety in humans must consider at least three dimensions: (1) exposure; (2) population at risk and end points; and (3) benefits versus cost and risk. The goal in this section will be to identify information that we have or do not have concerning these three basic dimensions of a comprehensive safety evaluation.

5.5.1 Exposure

The exposure conditions must also be considered in assessing the safety of ultrasound usage. As pointed out elsewhere in this document (section 4.1), the temporal peak intensities of pulsed ultrasound may be very high in comparison to the temporal average intensities. Therefore, the safety of pulsed ultrasound will have to be studied separately from that of continuous wave ultrasound.

Documented studies of the safety of ultrasound use on pregnant women are oriented toward device output rather than actual fetal exposure. This situation is understandable, because of the difficulty of quantifying fetal exposure. Intensity levels at the mother's skin and frequency of the sound waves are the parameters used to quantify exposure in existing studies. Frequency, intensity, duration, and number of exposures, fetal age at exposure, and possibly tissue specificity should be considered in studies of safety in humans. Output levels from diagnostic ultrasound equipment are discussed in section 4.1.

5.5.2 Populations at Risk and End Points

The ideal approach, to study a representative population for each possible combination of the dose factors discussed above, is unlikely. It might be possible to design a single investigation of a suitable study population at a selected number of exposure levels that would adequately characterize most obstetrical applications. If the manufacturing, regulatory, and academic communities can agree on the doses and populations to be studied, such a study, or studies, hold promise for contributing to our knowledge of the safety of most ultrasound applications.

5.5.2.1 Obstetrical Ultrasound

Populations used in studies of ultrasound have severely restricted the range of inferences that can be made concerning ultrasound safety in obstetrics. The possibility exists that ultrasound could affect abnormal pregnancies differently from normal ones.

Kohorn et al., (1967) examined 20 normal babies during the first 3 days of life. The examination consisted of an EEG recorded before and after application of an A-scan probe on the parietal or posterior-frontal region of the skull. A baseline 3-minute EEG was first done, then the transducer was activated for 10 minutes, after which a 5-minute EEG recording was made on each infant. No alteration in cerebral electrical activity was detected by visual examination of the tracings, but some infants showed poorly differentiated responses evoked by stimuli such as hand claps and flashes of light. These authors did not measure the intensities and frequencies of ultrasound exposures to which the infants were exposed, nor did they have a control group. This study of the newborn is included for completeness, even though the exposure to ultrasound is not typical of fetal exposure.

Bernstine (1969), studied the effects of exposure of 720 pregnant women to a Doppler examination. These women were either clinic patients with uncomplicated pregnancies, or were hospitalized with evidence of a threatened abortion or intrauterine fetal death or had some medical or obstetric complication of pregnancy. Although the two types of patients were described, the numbers in each group were not given and the two groups were not distinguished in the presentation of results. There were no controls in this study. It was difficult to tell whether this study had any predetermined end points. The indication for doing the Doppler examination and the end point of the study (fetal death) were apparently identical for one of the groups. It was not possible to discern which complications preceded and which followed the ultrasound examination. The author concluded that the incidence of obstetric complications was "similar in patients regardless of whether they had a Doppler study of their fetus or not." Since there was no unexposed population in this study, such a conclusion was unfounded.

Hellman et al. (1970) studied the fetal outcome of 1,114 pregnancies that were thought to be completely normal at the time of their ultrasound examinations. Most women were exposed to pulse-echo, some to continuous wave, and some to both types of ultrasound. These exposures occurred anywhere from 10 to 40 plus weeks of gestation, and patients had from 1 to more than 10 examinations. Besides the fact that the study involved only normal pregnancies, there was no control population for this study, making outcome assessment difficult. Furthermore, most data were reported on only 1,079 patients: the 35 patients who had spontaneous abortions were omitted from data analysis or were analyzed separately. This is unfortunate, as the cause of the abortions is unknown. The authors concluded that the abnormalities seen were no more common than in the general population. From details given in their tabulated results, it is apparent that only anatomical abnormalities were ascertained. They did not explicitly state when these abnormalities were diagnosed but presumably it was in the newborn period. Only 146 of the 1,079 newborn studied were exposed to ultrasound as early as 10 weeks of gestation. Such a small number exposed during the first trimester, coupled with an evaluation limited to anatomical abnormalities detectable in the newborn period, further limits the value of this study.

Falus et al. (1972), in Hungary, looked at gross developmental abnormalities in 171 children aged 6 months to 3 years who had been exposed to ultrasound in utero. Of the original 400 participants in this study, only 171 responded to a request for followup. More than one-third of the patients were born in a pathologic delivery which was related to the initial indication for ultrasound. The examinations of the children were "comprehensive"; however, cardiological, opthalmological, otolaryngological, orthopedic, and laboratory studies were carried out only "whenever it was necessary." Apparently no neurological examinations were done, but parents were asked to evaluate their child's emotional status. In addition, karyotypes from 10 exposed children and 10 control children were compared, but no increase in anomalies was seen. The authors concluded that bodily and mental development of the patients was at least average and sometimes better than average, and that no chromosomal effect could be shown from ultrasound. The major weaknesses of this study are that the patients were of a higher socieconomic status than the general population, there was a low followup rate with no attempt to characterize nonrespondents and there was no control population. In the case of the patients whose chromosomes were examined, the sample size was insufficient to detect meaningful results. End points were not evaluated to an extent that would justify the conclusions stated. Finally, almost all of the ultrasound examinations (78 percent) were done at more than 31 weeks gestation; only 3 percent were done between 8 and 12 weeks, the period when gross organ deformation would have been more likely to occur.

Scheidt et al. (1978), compared 297 infants of mothers who received both amniocentesis and ultrasound with a similar group of 661 infants whose mothers had had amniocentesis only and with 949 infants exposed to neither amniocentesis nor ultrasound. There was no group that was exposed to ultrasound only. The women exposed to both ultrasound and amniocentesis appeared to be of a higher socioeconomic status than the women who had amniocentesis only. The authors looked at 123 outcome variables, including those concerned with pregnancy, delivery, and the neonatal period. Followup data were collected at

approximately 1 year of age and included a reported history of certain health problems, a physical and neurological examination, and the Denver Developmental Screening Test. Subjects whose pregnancies terminated with induced abortion or with infants having genetic or metabolic problems diagnosable by amniocentesis were excluded from analysis. Although most patients exposed to ultrasound were exposed between 13 and 20 weeks, a time that organogenesis would not be likely to be affected, such outcomes should not be ruled out in the analysis. For grasp and tonic neck reflexes, there were more abnormalitites in the amniocentesis with the ultrasound group than in the group with neither. No such difference was seen between the ultrasound with amniocentesis and the amniocentesis only groups. Other differences seen between the three groups were not found to be statistically significant. The authors called for longer followup periods, more refined measures of outcome, and larger study populations.

Lyons, et al. (1980) has followed a population of 10,000 women exposed to pulsed ultrasound during pregnancy for the period 1970-1978. The authors report no statistically significant findings among the offspring of these women. However, an increase in the number of low birth weight infants was observed in those exposed in the second and third trimester when compared to a control population. This difference was not considered by the authors to be ultrasound associated.

Several studies have been done to examine the effect of ultrasound on human chromosomes. Watts & Stewart (1972) determined the frequency of chromosome aberrations in lymphocytes from 10 newborns and their mothers on whom fetal ultrasound monitoring was used during labor, and from 10 babies and mothers who received no insonation. Mothers and babies were exposed for 2 to 10 hours. At the time of delivery 5 ml of peripheral venous blood were taken from the mother and 5 ml of fetal blood were taken from the cord. The lymphocytes were cultured for 48 hours and fixed in colchicine. Slides were made and scored "blind" for chromosome aberrations; 100 cells were scored for each culture. Only the frequencies of ring and dicentric chromosomes were noted. No differences were found between the insonated and control groups.

Ikeuchi et al. (1973) exposed 98 pregnant women to continuous wave ultrasound at 40 mW/cm^2, 2 MHz, for 5 minutes. The control group of 103 pregnant women was not exposed. Immediately after the exposure, curettage was performed and chromosome observations were made, mostly on cultured fibroblasts of the fetal tissue. The mean gestational age was 9.53 $\pm$ 0.116 weeks in the exposed group and 9.80 $\pm$ 0.22 weeks in the control group at time of curettage. Slides were made of the cells and scored "blind" for anomalies. No difference was found in the proportion of cells with chromosome aberrations between the control (3.36 percent) and the exposed groups (3.39 percent); nor was there an apparent difference in the type of aberration between the two groups. As the authors pointed out, however, studies of this kind look at stable type aberrations and do not allow for aberrations that may be lost during adaptation to cell culture.

Abdulla et al. (1971) reported on the effect of diagnostic pulsed and continuous wave ultrasound on maternal and fetal chromosomes in 46 patients about to undergo therapeutic abortion. Twelve of the patients were exposed for 1 hour to pulsed ultrasound, 12 were exposed for 10 hours to a continuous wave fetal heart detector, 11 for 10 hours to a fetal heart monitor, and 11 patients were not exposed. The exposures were 1.5 to 2 MHz in frequency, and had average intensities of .8 to 22 mW/cm^2. Lymphocyte cultures were made of fetal and maternal blood and scored "blind" by two observers after one round of mitosis for chromosome breaks and gaps. No differences between the four groups were detected.

Serr et al. (1971) reported an increase in chromosome breaks in fetal cells from amniotic fluid exposed in vivo to 6 MHz ultrasound of intensity 22 mW/cm^2. In a later experiment using pregnant mice and a higher intensity of ultrasound, Mannor et al. (1972) did not find a difference in chromosomal aberrations between the control and experimental groups. These findings contradicted their previous human work (Serr et al., 1971).

In a clinical study of babies born to 150 mothers (Serr et al. 1971), one case of osteogenesis imperfecta was observed. This was the only neonatal death in the group. This case involved a 38-year-old mother who had been treated for prolonged secondary sterility. The woman was a physician's wife who repeatedly asked for reassurance by demanding to hear the baby's heart. This resulted in an estimated total exposure time of 8 hours between the twelfth and twenty-sixth week of gestation. The exposure time for all other cases did not exceed 15 minutes per patient. This case does not have any statistical significance with regard to a cause-effect relationship.

Studies on ultrasound have, for the most part, concentrated on physical and mental well being of the fetus and possible chromosomal aberrations that may be induced. Recently, two studies have emerged that investigated increased fetal movement in response to ultrasound. The first study, by David et al. (1975), looked at fetal movement in pregnant inpatients in response to Doppler monitoring. The patients were between 28 and 40 weeks gestation and hospitalized for various obstetric disorders. Using six to eight patients in each group, the investigators found that patients reported increased fetal movement when the ultrasonic transducer was activated compared to when it was not activated. The patients were unaware of the status of the activation of the machine, but the observers were, therefore, introducing a potential bias. The total observation time was 30 minutes. Hertz et al. (1979) found on the contrary no increased fetal movement in his population of 13 normal term patients when they were exposed to ultrasonic fetal monitoring. These experiments should be repeated with larger sample sizes, double masked (patient and observer) recordings of activated and inactivated monitors, and clear definitions of fetal movement.

The effects of ultrasound that have been discussed so far have concentrated on presumed direct effects. Indirect effects of use of the technology may also exist and in fact are of as much immediate concern as the direct effects. Fetal monitoring, in addition to exposing mother and child to ultrasound (and thereby possibly affecting future generations), may affect pregnancy outcome by confining a laboring woman to the supine position, by prolonging her labor, or by increasing the probability of Caesarian section and the concomitant increased maternal and infant morbidity. The psychological impact of the monitor on the laboring mother must also be taken into consideration.

5.5.2.2 Nonobstetrical Ultrasound

Ultrasound is used for a variety of nonobstetrical clinical purposes. Accordingly, a variety of tissues have been studied for ultrasound effects. For example, Sanada et al. (1977) exposed humans with 2.25 MHz ultrasound from a diagnostic cardiac pulse-echo device with an average intensity of 0.5 mW/cm^2. The authors determined the numbers of platelets that existed in different morphological groupings. They observed an initial shift during the first 15 minutes of exposure, with a tendency to return partially to normal thereafter. Thrombus formation was reported by Kahlert (1950) in a few patients in a followup study of individuals treated with therapeutic ultrasound. Because of the limitations of this study, it was not possible to determine if thrombosis was caused by factors other than ultrasound.

Ultrasound absorption produces heating of various internal structures such as joints, muscle and tendons (Lehmann et al., 1970; Lehmann & Guy, 1972; Warren et al., 1971). Tendons subjected to the same loads while maintained at normal tissue temperature exhibited little residual elongation. However, after a therapeutic application of heat (using ultrasound) and physical tension on the tendon permanent elongation has been achieved (Lehmann & Guy, 1972). Ultrasound therapy has been used in conjunction with stretching and motion exercises to assist in extending limited joint motion (Bierman, 1954; Lehmann et al., 1954, 1959, 1964). This technique has also been applied in the treatment of scar tissues and in instances where there has been a loss of joint function resulting from fibratic muscles and the disposition of collagenous tissues in the synovium of joints (Lehmann & Warren, 1974; Bierman, 1954).

Wound healing, an effect that claimed to be at least partially unrelated to temperature elevation of the tissue, has been reported by Dyson and colleagues (1968, 1970). This involved the stimulation of tissue regeneration with low-intensity pulsed and continuous wave ultrasound using temporal-average, spatial-average intensities on the order of 100 mW/cm^2 and above. Dyson first reported a stimulation of regeneration measured in terms of an increase in the area of regeneration of wounds in animals. Dyson also reported the use of low-level ultrasound on humans as a therapeutic application to increase the healing of chronic varicose ulcerated skin (Dyson et al., 1976). Because of the low intensity at which this effect was observed, it has been attributed to a mechanism other than heating (Lehmann & Guy, 1972: Dyson et al., 1968; Dyson, 1970). Dyson also reported increased effectiveness of the therapy by increasing temporal peak power while maintaining the same temporal average power. The treatment of chronic skin ulcerations with ultrasound has also been reported by others (Paul et al., 1960; and Galitsky & Levine, 1964).

5.5.3 Benefits Versus Risks

Choosing end points for study is especially difficult in human subjects. Latent periods easily could be as long as 20 years in the case of cancer development, or the effect may not be seen for another generation. The effects of ultrasound at diagnostic levels cannot necessarily be predicted from what we know to be the effects of therapeutic levels. Because the human fetus is sensitive to other forms of radiation (Brent, 1980), there is considerable concern that it may also be sensitive to ultrasound. Thus, looking at prenatal exposure is a prudent step in investigating human effects. Until now, the fetus has been the focus of our concern; however, exposure of the mother could pose an equally or more significant risk.

Animal studies suggest neurologic (sensory, cognitive, and developmental) Murai et al., 1975a, 1975b; Sikov et al., 1976; Sikov & Hildebrand, 1977), immunologic (Anderson & Barrett, 1979; Bases, 1979), and hematologic (5.4.4.1) possibilities for study in humans. There is some evidence (Shoji et al., 1971; Sikov et al., 1976) that if exposure is within the period of organogenesis, congenital malformations may result from exposure to ultrasound in laboratory animals. In general, these end points in animal studies have been unexplored in humans and should be followed up wherever possible. Further, it must be realized that animal studies may not have explored all possible adverse effects, and it is quite possible that animal studies will not reveal some potential problems in humans.

It is not clear at this time whether ultrasound fetal monitoring is beneficial to the mother or fetus in terms of pregnancy outcome and this, above all, should be examined closely; if there is no generally acknowledged benefit to the monitoring, there is no reason to expose patients to increased costs and possible risk. Similarly, such a benefit and risk ratio must be considered for diagnostic ultrasound used in early pregnancy. This technique's benefits are well accepted for determining gestational age, placental position for amniocentesis, certain congenital malformations, multiple pregnancy, and the position of the fetus. It is also being increasingly used in a routine manner to provide the physician with a complete "picture" of the pregnancy. The benefits associated with such use must be compared with the associated cost and possible risk to mother and child.

In the final analysis, end points may be viewed as benefits versus risks. If major benefits are not gained by a certain procedure, yet risks are incurred, this may be sufficient cause to discontinue a common practice. Similarly, if major benefits are gained, but at the expense of possible risk, a patient and the physician may elect to proceed with the procedure indicated. At the moment, no well documented direct risks of the diagnostic use of ultrasound in humans are established. Indirect risks have been suggested for fetal monitoring, as has been described previously, yet the question of benefit has not been resolved. Assessment of the "safety" of ultrasound requires that much new work be done.

5.5.4 Research Gaps

To date, no effort has been made to study a representative obstetric population, consisting of an acceptable variety of population subsets. Convenience, rather than a systematic approach, has governed the method used. In future studies efforts to include adequate representation of the following dimensions should be made: pregnancy complications, gestational age at the time of exposure, maternal age, socioeconomic status, ethnic origin, and geographic location. Two on-going epidemiologic studies attempt to control for some of these factors (Moore et al., 1982; Lyons et al., 1980).

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6. SUMMARY AND CONCLUSIONS

6.1 SUMMARY: DIAGNOSTIC ULTRASOUND

In recent years, there has been widespread adoption of ultrasound procedures in diagnostic medicine, particularly in obstetrics, cardiology, and radiology. At present, there is no clearly established evidence to assess the risk involved with ultrasound diagnostic exposures. This section will discuss and summarize the biological effects data discussed in detail in previous sections with reference to human health risk.

A variety of topics that have been detailed in section 5 are examined below. Since evidence from human exposure provides the most direct evidence of biological risk from ultrasound, this will be considered first. In addition to human data, it is useful to examine data from experimental studies. These have been discussed in detail in the earlier sections and deal with developmental, hematological/vascular effects and effects on genetic material. There are also some data concerning immunological and neurological/behavioral effects. In addition, cumulative and synergistic effects are discussed.

To date, very little information from adequately controlled human epidemiological studies is available. Not surprisingly, then, the current data from human experience is inconclusive. However, since ultrasound has been used for several years with no obvious detrimental effects, we can be reasonably confident that dramatic, acute effects are not likely. On the other hand, studies designed and conducted to detect less obvious effects (such as those associated with low levels of ionizing radiation) have not been performed. Therefore, the question of subtle and/or long-term effects remains unanswered. Definitive estimates of risk cannot be provided by a single study and it will be many years before substantial evidence is obtained from human exposure. However, recently reported data on human effects raises concerns about human exposure at diagnostic levels (Moore et al., 1982).

The current information from controlled laboratory experiments, although incomplete, indicates several areas of concern. Although many of the studies do not mimic clinical exposure conditions precisely, there are several reports of effects within the range of intensities that have been measured from clinical diagnostic devices. Of particular concern are those studies (section 5.4.1) which indicate that ultrasound can induce developmental effects. This concern stems from the extent of use of ultrasound devices during pregnancy and the recognized susceptibility of embryonic tissue to a variety of insults.

Several studies that were described in section 5.4 indicate that low intensities of ultrasound can produce hematological and vascular alterations in vitro and in vivo. The possibility that such effects could represent a health hazard to individuals already predisposed to abnormal hematological or vascular conditions should be thoroughly investigated, particularly at the higher intensities used in ultrasonic therapy and in diagnostic investigations involving long exposure times such as in Doppler fetal monitoring.

The effects of ultrasound on genetic material observed to date have been largely discounted by many investigators because of peculiarities of the test systems. Degradation of DNA in solution is often attributed to the occurrence of cavitation within the solution. It should be noted that stable cavitation has been demonstrated in mammalian tissue at cw intensities as low as 80 mW/cm^2 (ter-Haar & Daniels, 1981).

Studies in vitro indicate possible effects on DNA of cultured mammalian cells exposed to pulsed clinical diagnostic ultrasound devices (Liebeskind et al. 1979a, 1979b; Fung et al., 1978; Prasad et al., 1976; Haupt et al., 1981). Exposure to diagnostic levels of pulsed ultrasound has been reported to elicit unscheduled DNA synthesis (Liebeskind, 1979a)

altered immunoreactivity of DNA to antinucleotide antibody (Liebeskind, 1979b) and increases in the frequency of sister chromatid exchanges (Liebeskind 1979b; Haupt et al., 1981). The implications of these in vitro results for human health are not known. For other types of insults sister chromatid assay has been suggested to be a sensitive measure of genetic damage, because the frequency of exchanges increased after exposure of cells to known mutagens and carcinogens (Stetka & Wolff, 1977). Another effect observed in vitro which raises questions about the possible significance and mechanisms involved is the inherited effect on cells exposed in vitro to pulsed diagnostic ultrasound showing a persistence of disturbances in cell motility many generations after insonation (Liebeskind, 1981).

There are some limited data suggesting immunological effects from ultrasound exposures both at the diagnostic and therapeutic levels (Anderson & Barrett, 1979, 1981). There is also limited information suggesting neurological and behavioral effects on rodents (Murai et al., 1975; Sikov & Hildebrand, 1977). However, the data in these two areas are insufficient to allow a definitive assessment.

The questions of whether the health effects can be cumulative, additive, or synergistic are of vital importance in assessing its safety. Although there have been conflicting reports of synergism between ultrasound and x rays in the destruction of tumors (Clarke et al., 1970; Woeber, 1965) there have been unrefuted reports that ultrasound and x rays reduce the electrophoretic mobility of tumor cells in vitro in an additive manner (Repacholi et al., 1971) and that ultrasound can enhance the x-ray induced inactivation of cultured hamster cells (Todd & Schroy, 1974). It also has been demonstrated that hypoxia and ultrasound exhibit synergisms by decreasing the exposure time required to produce hemmorhage in the rat spinal cord (Taylor & Pond, 1972). As in other areas of concern, the available data are insufficient to allow a definitive assessment of the accumulative, additive, or synergistic nature of ultrasound effects.

Reviews of the available biological effects data have been prepared by various investigators (Ulrich, 1974; Wells, 1973; Nyborg 1978; Fry, 1979; Sarvazyan, 1981). The American Institute of Ultrasound in Medicine (August 1976; revised October 1978) has endorsed the following statement:

> "In the low megahertz frequency range there have been (as of this date) no independently confirmed significant biological effects in mammalian tissues exposed to intensities* below 100 mW/cm^2. Furthermore, for ultrasonic exposure times** less than 500 seconds and greater than one second, such effects have not been demonstrated even at higher intensities, when the product of intensity* and exposure times** is less than 50 joules/cm^2."

Comments prepared by the American Institute of Ultrasound in Medicine, Bioeffects Committee regarding the above statement appear in Appendix III. In the practical application of this statement, the spatial peak values of intensity referred to in the statement should not be confused with the spatial-average, temporal-average values of intensity sometimes used for the specification of ultrasound exposure (Hill, 1980). The former value in practice can exceed the latter by a factor of 2 to 6 for unfocused transducers and sometimes much more for focused transducers (Hill, 1980).

These curves and analyses have been widely interpreted as representing a "safe" level of diagnostic ultrasound. Such an interpretation disregards the fact that the data representing primarily prompt responses to exposure are a collation of isolated data points from numerous

*Spatial peak, temporal average as measured in a free field in water. The spatial peak intensity should be determined with a device, such as a calibrated miniature hydrophone, for which the dimensions of the sensitive area are smaller than the distance over which the local value of the ultrasound field intensity shows a significant variation.

**Total time; this includes off-time as well as on-time for the repeated pulse regimen.

nonsystematic studies. Also "thresholds" tend to decrease as more sensitive end points are studied and more sensitive means for measuring the end points are used. In fact, there are several recent studies (Sanada et al., 1977; Liebeskind et al., 1981; Sarvazyan 1981; Anderson & Barrett, 1981; Moore et al., 1982) reporting effects at exposures below any of the aforementioned curves. Sarvazyan (1981) in a review of the biological action of ultrasound calls into question the use of a boundary line defined by the product of intensity (I) and time (t) (i.e., It = 50 J/cm^2) as is done in the Nyborg analysis and the AIUM statement. There is evidence that under some conditions effects may be related to the temporal peak intensity or other parameters associated with pulsed diagnostic ultrasound (see sections 2.6, 5.1, and 5.4.1). Although graphical analysis of isolated biological effects data do not define a safe region, they do suggest a potentially hazardous region.

6.2 SUMMARY: THERAPEUTIC ULTRASOUND

In the preceding discussion of health risks associated with diagnostic ultrasound, we have examined developmental, hematological/cardiovascular genetic, immunological and neurologic/behavioral effects. Clearly, at the even higher cw exposure levels associated with therapeutic ultrasound, similar considerations would apply. However, at therapeutic levels, we must also consider the effects on skeletal and soft tissue that would not be of concern for the lower levels associated with diagnostic ultrasound.

Since fetal abnormalities and reduced pup weight have been observed in the offspring of pregnant mice exposed to intensities in the range of those used in therapy (see section 5.4.1), pregnant women should not receive ultrasonic therapy in the region of the uterus.

In a study of the possible consequences of using unfiltered waveforms, Lehmann et al., (1953, 1970) concluded that a potentially dangerous situation due to the temporal peaks could result from a lack of line voltage filtering if only the temporal average power and temporal average effective intensity were known. In considering this information, it seems apparent that if one wishes to achieve primarily thermal effects, continuous wave ultrasound would be a prudent choice in order to minimize any risks associated with the temporal peaks.

Ultrasound exposure over a strong reflecting surface, such as bone, may result in the formation of standing waves that could produce a blood-flow stasis effect (Dyson et al., 1971, 1974). Endothelial damage to the blood vessels may occur if this stasis effect occurs for an extended period of time (Dyson, 1974).

When treating peripheral vascular disease, the patient may not detect overexposures to ultrasound within extremeties because of diminished sensation and lack of blood circulation.

High temperatures resulting in periosteal pain are potentially dangerous and the therapist should respond to any complaint of patient pain and adjust the treatment accordingly. At levels higher than normally employed in physical therapy, damage to the bones of experimental animals has been reported (Janes et al., 1962).

The reported effects of therapy levels of ultrasound on growing bones raise serious questions about possible effects if the epiphyseal lines in children are to be exposed to ultrasound, especially when these areas are still in the growing stages (Kolar et al., 1964).

6.3 SUMMARY: DENTAL APPLICATIONS

The primary therapeutic use of ultrasound in dentistry is for "scaling" or removal of calculus. Adverse effects are a serious possibility if this is improperly done. The best known effect is due to heating. Modern ultrasonic scaling devices have a water spray or mist for cooling the tool tip and tissue interface. This same water mist obscures vision at the scaling site, thus optimum water flow adjustment is needed (Frost, 1977). Too much

water hinders operation and possibly drives dislodged calculus into the gingiva; too little leads to tip heating and patient and operator discomfort. It is also necessary that the instrument tip be kept in constant motion to avoid unnecessary "spot" heating of the teeth (Johnson & Wilson, 1957; Jarabak, 1961).

Scratching or "gouging" of teeth by ultrasonic scalers is well documented (Forrest, 1967; Johnson & Wilson, 1957; Moskow & Bressman, 1964; Ewen, 1966; Wilkinson & Maybury, 1973). The training and experience of the operator are a significant factor in the occurrence of this effect. For example, at the beginning of a training course for dental hygienists, nearly all the surfaces of the artificial teeth scaled showed "considerable scratching" whether by hand or ultrasound procedure. Toward the end of the apparently 1-year long course, teeth showed little or no evidence of this (Forrest, 1967).

6.4 CONCLUSION: DIAGNOSTIC ULTRASOUND

In conclusion, many deficiencies and gaps exist in the current data base for ultrasound-induced bioeffects. The potential for acute adverse effects has not been systematically explored and the potential for delayed effects has been virtually ignored. Few of the experiments have been replicated in other laboratories. More information is needed on the effects of low-intensity ultrasound, the biological impact of the high temporal intensities used in pulsed ultrasound, the possibility of cumulative effects, and the possibility of long-term effects. Most current information is based on laboratory studies and the clinical significance of the information is not clear. However, based on current diagnostic usage and available research information, the following are general areas of concern in which additional research is desirable: developmental effects (including behavioral and neurological), hematologic and immunologic effects, and genetic effects. Although the body of current evidence does not indicate that diagnostic ultrasound represents an acute risk to human health, it is insufficient to justify an unqualified acceptance of safety.

Because the assessment of potential risks from exposure to low levels of chemicals, radiation, or other forms of energy is difficult, it is unreasonable to expect that in the near future, the degree of risk will be clearly defined for diagnostic ultrasound. No single study, epidemiologic or experimental, can accomplish this goal. A systematic investigative effort involving in vitro, animal and human studies to define the effects of ultrasound and the mechanisms underlying these effects will be required.

In the meantime, actions and recommendations can and should be taken on current data. These should be revised as more data become available. Until risks are better defined, a prudent public health policy dictates judicious use of diagnostic ultrasound. Ultrasound examinations should be used only when diagnostic benefits to patients are indicated, and the exposure duration and ultrasound intensity should be limited to that required to yield the necessary diagnostic information. The FDA has previously enunciated this in some general recommendations (FDA Notice of Intent, 1979).

Canada and Japan have both taken steps to minimize exposure via equipment standards and guidelines. The Canadian safe use guidelines recommend that diagnostic ultrasound equipment producing SATA intensities above 100 mW/cm^2 should have instruments for monitoring both exposure level and duration of exposure (Guidelines for the Safe Use of Ultrasound, 1980) and a draft Japanese standard requires an upper limit of 10 mW/cm^2 for the SATA intensity for Doppler ultrasonic fetal diagnostic equipment (Japanese Industrial Standards, 1979).

The development of guidelines for patient referral in addition to recommendations for equipment performance would assist appropriate utilization of the modality.

6.5 CONCLUSION: THERAPEUTIC ULTRASOUND

Ultrasound therapy is intended to elevate the temperature of the exposed tissue and cause a salutory effect. If the temperature elevation is excessive, the resulting effect can be tissue destruction. For this reason, the development of criteria for application of ultrasound therapy would facilitate good patient care.

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7. RECOMMENDATIONS FOR FURTHER RESEARCH

A number of areas needing further research are identified in this review including measurement of ultrasonic fields, development of instrumentation, mechanisms of interaction, as well as studies on molecules and cells, and developmental, hematological/cardiovascular, genetic, immunological, behavioral, and human effects. The need for research in these areas is discussed in the remainder of this chapter. In general, it should be noted that a substantial systematic research effort is required to confirm or refute reports of the number of biological effects already identified and to pursue those areas that show promise in identifying biological effects and the mechanisms by which ultrasound produces these effects. These recommendations are not listed in order of priority.

7.1 MEASUREMENT OF INTERNAL ULTRASONIC FIELDS

The spatial and temporal characteristics of ultrasound fields radiated by clinical devices into water is relatively well established. Fundamental characteristics of ultrasound tissue interactions, such as attenuation, velocity, and impedance, have been measured but because of differences in these values from one tissue to the next and because of differences in the geometry of experimental animals, and the anisotropic nature of the acoustic properties of many biological materials, this information is difficult to use in obtaining reliable estimates of internal exposure fields. Therefore additional work is needed to develop instrumentation and techniques capable of measuring internal exposure parameters. In the development of these detectors, interaction mechanisms are likely to be an important design consideration. For example, a detector that measures absorbed energy would be useful for quantifying effects that are due to thermal mechanisms, but a detector that responds to peak pressure amplitude may be more useful for quantifying cavitation phenomena. Much work is required in this area including the development of adequate phantoms.

7.2 INSTRUMENT DEVELOPMENT AND MEASUREMENTS

With regard to clinical instrumentation there is a dual need. First, instrumentation should be developed to provide methods of measuring the exposure levels from diagnostic ultrasound equipment. Instruments and methods for field measurement of the temporal and spatial intensities need to be developed. Second, there must be more complete knowledge of the intensities to which patients are exposed by currently available devices and the many new devices being introduced each year.

7.3 STUDIES OF MECHANISMS OF ACTION

The degree of importance of various physical mechanisms in producing biological effects needs systematic investigation. At higher intensities, the absorption of ultrasonic energy by conversion to heat is well recognized as a mechanism causing biological effects. Not so thoroughly examined have been nonthermal interactions such as cavitation and viscous stresses. The latter includes microstreaming and shear stresses, radiation forces, radiation torques, and stresses associated with particle acceleration.

7.4 IN VITRO STUDIES

Investigations at the level of macromolecules and cells offer promise as a screening technique to evaluate the relative importance of various physical mechanisms of interaction and clarify the need for further study at higher levels of biological organization.

Alterations to cell membrane structure have been reported by a number of investigators. Some alterations include increased density of microvilli and ruffles in cell membrane following exposure that may alter growth characteristics. Studies are needed to establish the mechanisms causing these effects and their potential significance. The persistence of hereditable disturbances in cell motility after ultrasound exposure is especially important and investigations need to be conducted to determine if these effects occur in vivo.

7.5 DEVELOPMENTAL STUDIES

Animal studies have shown potential effects on fetuses, such as fetal weight reduction and cardiac defects and other fetal anomalies in rodents, following in utero exposure. Some of the reported effects, such as fetal abnormalities have been questioned and further work is needed to establish cause and effect. Studies are needed to determine the relative biological importance of the high temporal peak intensities and other parameters associated with pulse-echo diagnostic equipment.

7.6 HEMATOLOGIC STUDIES

Investigators have reported effects both in vivo and in vitro at diagnostic and therapeutic intensities suggestive of perturbations of the hematatologic system in laboratory animals and in human patients. Systematic research should be conducted to thoroughly investigate reported effects such as formation of thrombi which could have adverse consequences in vivo, effects on platelets and increased fragility of blood cells after long exposure, such as that involved in fetal monitoring.

7.7 GENETIC STUDIES

Although hereditable genetic effects from ultrasound have not been demonstrated in vivo, additional investigations are needed not only to substantiate or refute some reported effects on genetic material, such as increased sister-chromatid exchange frequency, unscheduled DNA synthesis, and alterations to nucleic acid percursor uptake but also to determine their significance. For example, for other types of insults sister-chromatid assay has been suggested to be a sensitive measure of genetic damage, because the frequency of exchange increases after exposure of the cell to known mutagens and carcinogens. Of particular importance are investigations of effects from high peak intensities and other parameters associated with diagnostic ultrasound equipment as compared to potential effects that might be caused by the lower time average intensities from continuous wave exposures.

7.8 IMMUNOLOGIC STUDIES

These studies suggest that ultrasound may affect immunologic responses in laboratory animals and in humans. Considering the fundamental importance of the immune system, it is important that these effects be confirmed or refuted. Investigation of possible effects of ultrasound on the immune system could have applicability in both the diagnostic and therapeutic areas.

7.9 BEHAVIORAL STUDIES

Some experiments with rodents suggest that behavioral effects may be shown by neonates exposed in utero. This has proven to be a difficult area of research because of problems in obtaining reproducible experimental results. If some of these difficulties can be overcome this may prove to be a very fruitful area of research for identifying potential consequences to the human fetus exposed in utero.

7.10 HUMAN STUDIES

Retrospective and prospective investigations are needed to observe effects on both mother and newborn exposed in utero and appropriate long-term followup should be conducted. An added difficulty to these types of studies is that control populations of unexposed neonates are rapidly disappearing as the use of ultrasound diagnosis increases. This fact provides further incentive toward prompt initiation of these studies. The importance of studies on the fetus is even more urgent since the introducton of ultrasound exposure to developing Graffian follicle for ovulation timing for in vivo fertilization. Short-term studies with specific end points, such as hematological and immunological investigations, should also be conducted.

APPENDIX I

GLOSSARY OF TERMS, UNITS, AND RELATIONSHIPS

Units, Relationships, and Conversion Factor

GLOSSARY OF TERMS AND UNITS

Term	Units	Symbol	Equivalent units or comment
Angular frequency	hertz (Hz)	ω	$\omega=2\pi f$
Power	watt (W)	P	J/s
Propagation constant	cm^{-1}	k	$2\pi/\lambda$
Intensity	watts/sq.meter (W/cm^2)	I	*
Speed of sound in the medium	meters/second (m/s)	c	
Force	newton (N)	F	kg/s^2
Pressure	pascal (Pa)	p	N/m^2
Particle displacement	meter (m)	s	
Particle velocity	meters/second (m/s)	v	
Particle acceleration	meter/sq.second (m/s^2)	a	
Characteristic acoustic impedance	rayls (r)	Z	$kg/(s^1m^2)$
Amplitude attenuation coefficient	nepers/cm	α	8.686 dB/cm
Amplitude absorption coefficient	nepers/cm	α_a	8.686 dB/cm
Intensity attenuation coefficient	nepers/cm	μ	2α
Wavelength	meter (m)	λ	
Frequency	hertz (Hz)	f	1/s
Energy	joule (J)	E	N•m (= W•s)
Density	kg/m^3	ρ	
Area	sq.meters (m^2)	A	

*In practice, it is more convenient to express intensity in units of watts per square centimeter (W/cm^2) or milliwatts per square centimeter (mW/cm^2).

The various relationships between the above parameters for a plane continuous monochromatic ultrasound field in a homogeneous lossless medium are given by the following relationships.

RELATIONSHIP OF TERMS

Particle Displacement

$$s = s_o \sin(\omega t - kx)$$

where s_o = maximum displacement amplitude
$\omega = 2\pi f$ = angular frequency
$k = 2\pi/\lambda$ = wave number
s = displacement amplitude

Particle velocity

$$v = \partial s/\partial t = v_o \cos(\omega t - kx)$$

where $v_o = \omega s_o$ = velocity amplitude
$k = 2\pi/\lambda$ = propagation constant

Particle acceleration

$$a = \partial v/\partial t = -a_o \sin(\omega t - kx)$$

where $a_o = \omega^2 s_o$ = acceleration amplitude

Acoustic pressure

$\partial p/\partial x = -\rho a$, hence

$$p = p_o \cos(\omega t - kx)$$

where $p_o = \frac{\rho\omega^2}{k} s_o = \rho c \omega s_o$ = pressure amplitude
c = propagation velocity

Specific acoustic impedance

$Z = p_o/v_o$, hence
$Z = \rho c$

Energy density

The total kinetic energy of a single oscillating particle (at maximum velocity) is
$e = mv_o^2/2$
m = mass of the particle
v_o = particle velocity amplitude

hence the energy density (E) of the sound field is
$E = \rho v_o^2/2$ or, using $Z = p_o/v_o = \rho c$
$E = \rho p_o^2/2Z^2 = p_o^2/^2\rho c^2$
ρ = mean density of the medium

Intensity

Intensity is equal to the speed of sound in the medium (c), times the total energy
$I = cE$
hence, using $E = p_o^2/2\rho c^2$
$I = p_o^2/2\rho c$

For a given intensity, the quantities s_o, v_o, a_o and p_o can be calculated from

$s_o = 1/\omega\,(2I/\rho c)^{1/2}$
$v_o = (2I/\rho c)^{1/2}$
$a_o = \omega(2I/\rho c)^{1/2}$
$p_o = (2\rho c I)^{1/2}$

The above relationships are based on the assumption of a plane continuous (sinusoidal) wave, and I represents the intensity of the wave averaged over one cycle. In such a wave, the instantaneous peak intensity (I_p) is twice the average value, i.e., $I = I_A$ and $I_p = 2I_A$.

If the wave consists of short asymmetric pulses, such as those emitted by pulse-echo diagnostic ultrasound instruments, it is usually not possible to define an average over one cycle. It is therefore necessary to specify the output of such instruments in terms of the instantaneous peak intensity,

$$I_p = p_o^2/\rho c$$

and the particle parameters are now given by

$$s_o = 1/\omega\,(I/\rho c)^{1/2}$$
$$v_o = (I/\rho c)^{1/2}$$
$$a_o = \omega(I/\rho c)^{1/2}$$
$$P_o = (\rho c I)^{1/2}$$

In Table A1, particle parameters for typical medical diagnostic instruments are given in terms of the average intensity (therapeutic and cw Doppler instruments) or the peak intensity (pulse-echo instruments).

Table A1. Particle parameters for typical frequencies and intensities generated by medical ultrasonic equipment

	Therapeutic ultrasound I_A = 100 to 3000 mW/cm^2 f = 1.0 MHz (cw)	Diagnostic ultrasound pulse echo I_p = 100 to 100,000 mW/cm^2 center freq. = 2.25 MHz	Diagnostic ultrasound cw Doppler I_A = 1 to 20 mW/cm^2 f = 2.25 MHz (cw)
Displacement amplitude (m)	5.8×10^{-9} to 3.2×10^{-8}	1.8×10^{-9} to 5.8×10^{-8}	2.6×10^{-10} to 1.2×10^{-9}
Particle acceleration (m/s^2)	2.3×10^{5} to 1.3×10^{6}	3.7×10^{5} to 1.2×10^{7}	5.2×10^{4} to 2.3×10^{5}
Acoustic pressure (N/m^2)	5.4×10^{4} to 2.9×10^{5}	3.8×10^{4} to 1.2×10^{6}	5.4×10^{3} to 2.4×10^{4}
Particle velocity (m/s)	3.7×10^{-2} to 2.0×10^{-1}	2.6×10^{-2} to 8.2×10^{-1}	3.7×10^{-3} to 1.6×10^{-2}

CONVERSION TABLES (* INDICATES EXACT CONVERSION)

Length

Multiply	by	to obtain
angstrom (A)*	1.000×10^{-10}	meter
micron*	1.000×10^{-6}	meter
inch*	$2.540 \times 10^{+1}$	millimeter
foot*	3.048×10^{-1}	meter
yard*	9.144×10^{-1}	meter

Area

square meter	$1,000 \times 10^{4}$	sq. centimeter
square inch	6.452×10^{2}	sq. millimeter
square inch	6.452×10^{-4}	sq. meter
square foot	9.290×10^{-2}	sq. meter
square yard	8.261×10^{-1}	sq. meter
acre	4.047×10^{-3}	sq. meter
square mile (int'l)	2.590×10	sq. kilometer

Volume

cubic inch	1.639×10^{-5}	cubic meter
cubic foot	2.832×10^{-2}	cubic meter
cubic yard	7.646×10^{-1}	cubic meter
liter*	1.000×10^{-3}	cubic meter
quart (US liquid)	9.464×10^{-4}	cubic meter
quart (US liquid)	9.464×10^{-1}	liter
gallon (US liquid)	3.785×10^{-3}	cubic meter
gallon (UK liquid)	4.546×10^{-3}	cubic meter
ounce (US liquid)	2.957×10^{-2}	liter
ounce (UK liquid)	2.841×10^{-2}	liter

Mass

grain	6.480×10^{-2}	gram
carat (metric)*	2.000×10^{-1}	gram
ounce (avdp)	$2.835 \times 10^{+1}$	gram
pound (avdp)	4.536×10^{-1}	kilogram

Force

dyne*	1.000×10^{-5}	newton
kilogram (force)	$9.807 \times 10^{+0}$	newton
ounce (force)	2.780×10^{-1}	newton
pound (force)	$4.448 \times 10^{+0}$	newton

Pressure

atmosphere (normal)	$1.013 \times 10^{+5}$	pascal
bar*	$1.000 \times 10^{+5}$	pascal
newton/sq. meter*	1.000	pascal
dyne/sq. centimeter*	1.000×10^{-1}	pascal
inch of water (4 °C)	$2.491 \times 10^{+2}$	pascal
mm of Hg (0 °C)*	$1.333 \times 10^{+2}$	pascal
pound /sq. foot	$4.788 \times 10^{+1}$	pascal
pound/sq. inch*	$6.895 \times 10^{+3}$	pascal

Multiply	by	to obtain
Velocity		
foot/minute*	5.080×10^{-3}	meter/second
foot/second*	3.048×10^{-1}	meter second
inch/second*	2.540×10^{-2}	meter/second
knot (kt)	5.144×10^{-1}	meter/second
mile (int'l)/hour	$1.609 \times 10^{+0}$	kilometer/hour
mile (int'l)/hour	4.470×10^{-1}	meter/second
Acceleration		
acceleration of gravity	$9.807 \times 10^{+0}$	meter/second^2
inch/second^2*	2.540×10^{-2}	meter/second^2
foot/second^2*	3.048×10^{-1}	meter/second^2
Angle		
cycle (360°)	$6.283 \times 10^{+0}$	radian
degree	2.745×10^{-2}	radian
Hertz	$6.283 \times 10^{+0}$	radian/second
RPM	1.047×10^{-1}	radian/second
RPS	$6.283 \times 10^{+0}$	radian/second
Energy		
btu (int'l table)	$1.055 \times 10^{+3}$	joule
calorie (int'l table)	$4.187 \times 10^{+0}$	joule
erg*	1.000×10^{-7}	joule
ev	1.6×10^{-17}	joule
watt-hour*	$3.600 \times 10^{+3}$	joule
ft-lb	$1.356 \times 10^{+0}$	joule
Intensity		
watts/sq. centimeter	10,000	watts/sq. meter
watts/sq. centimeter	1000	milliwatts/sq. centimeter
watts/sq. meter	0.0001	watts/sq. centimeter
watts/sq. meter	0.1	milliwatts/sq. centimeter
Power		
btu (int'l table)/hour	2.931×10^{-1}	watt
hp (electric)*	$7.460 \times 10^{+2}$	watt
erg/sec*	1.000×10^{-7}	watt
cal/sec* (thermochemical)	$4.184 \times 10^{+0}$	watt
Attenuation		
dB	0.115	neper
neper	-8.686	dB
Temperature		
Celsius to Kelvin	°K = °C + 273.15	
Fahrenheit to Celsius	°C = (°F-32)/1.8	
Fahrenheit to Kelvin	°K = (°F + 459.67°)/1.8	
Rankine to Kelvin	°K = °R/1.8	

Unit prefixes -	Prefixes -	mega,	kilo,	centi,	milli,	micro
	Factor* -	1,000,000,	1,000,	0.01,	0.001,	0.000001
	Symbol -	M,	k,	c,	m,	μ,

*Factor is the number of unprefixed units in a unit with the prefix. For example, there are 1,000 Hz in 1 kHz, and 3 is 0.001 meters in 1 millimeter.

DECIBEL NOTATION

The need often arises for comparing intensity or pressure levels; for example, when considering the amount of reflection or absorption which an ultrasonic field has undergone. In this case, it may be an advantage to express the ratio of intensities or pressures as a logarithm because this affords a simple method of expressing numbers that extends over many orders of magnitude and because the arithmetic product of two or more quantities are obtained by the addition of the logarithms. The decibel (dB) is the logarithmic unit most commonly used for expressing the ratio of two quantities.

It is important to realize that since the decibel unit is only a relative measurement, a reference level must also be stated to obtain an absolute value. For example, an intensity of 16 dB above 1 W/cm^2 is equal to 39.81 W/cm^2. A reference level usually used for audible sound is 20 micropascals which corresponds to the weakest sound a human being can hear.

The formulae for the decibel and reference levels sometimes used are as follows:

DECIBEL FORMULAE AND REFERENCE LEVELS SOMETIMES USED

Quantity	Formula	Reference level*
Sound pressure	20 log (p/p_o) dB	20 μPa (in air) 1 μPa (in other media)
Acceleration	20 log (a/a_o) dB	1 $\mu m/s^2$
Velocity	20 log (v/v_o) dB	1 nm/s
Force	10 log (F/F_o) dB	1 μN
Power	10 log (P/P_o) dB	1 pW
Intensity	10 log (I/I_o) dB	1 pW/m^2
Energy density	10 log (W/W_o) dB	1 pJ/m^3
Energy	10 log (E/E_o) dB	1 pJ
Voltage	20 log (V/V_o) dB	

*p_o, a_o.....

Note that a change of 3 dB changes power, intensity, force, and energy by a factor of 2, and sound pressure, acceleration, velocity, and voltage by a factor of the square root of 2 or 1.41. A change of an additional 3 dB to make 6 dB represents a power ratio of 2^2 or 4 and sound pressure a ratio of 2.

In biological effects literature, reference is often made to the neper. The neper is a logarithmic unit corresponding to a reduction in amplitude (e.g. pressure amplitude) to 1/e of the initial value, i.e.,

$$\text{nepers} = \log_e (P/P_o)$$

Since this corresponds to a change of -8.686 dB, we can say that 1 neper = 8.686 dB.

APPENDIX II

Definition of Terms - The following is a list of terms related to the measurement and calibration of medical ultrasonic equipment. These definitions are based on ones used in the AIUM/NEMA Safety Standard for Diagnostic Ultrasound Equipment (1981) or the U.S. Ultrasonic Therapy Products Radiation Safety Performance Standard (1978). Defined terms are underlined.

1. Acoustic pressure - means the value of the total pressure minus the ambient pressure.

2. Amplitude modulated waveform - means a waveform in which the amplitude modulation factor is greater than 5 percent.

3. Amplitude modulation factor - means the value of the expression $100 \times (|A| - |B|)/|A|$ where A and B are the absolute maximum and minimum values, respectively, of the envelope of a modulated acoustical or electrical carrier (first order quantity), expressed as a percentage.

4. Bandwidth - means the difference in the frequencies f_1 and f_2 at which the transmitted acoustic pressure spectrum is 71 percent (-3 dB) of its maximum value.

5. Beam axis - means a straight line joining the points of maximum pulse intensity integral measured in the far field. This line, calculated according to regression rules, should be extended back to the transducer assembly surface.

6. Beam cross section - means that surface in any plane consisting of all the points at which the intensity is greater than X % of the spatial maximum intensity in that plane. For beams from therapy equipment X is usually 5 percent; for ultrasonic fields from diagnostic equipment X is usually 25 percent, and the plane is taken to be perpendicular to the beam axis.

7. Beam cross sectional area - means the area of the beam cross section. In the AIUM/NEMA Standard beam cross sectional area means the area on the surface of a plane perpendicular to the beam axis consisting of all points where the pulse intensity integral is greater than 25 percent of the maximum pulse intensity integral in that plane. For situations in which the relative waveform does not change significantly across the beam cross sectional area, the beam cross sectional area may be approximated by measuring the area on the surface of a plane perpendicular to the beam axis consisting of all points where the acoustic pressure is greater than 50 percent of the maximum acoustic pressure in the plane.

8. Beam nonuniformity ratio - means the ratio of the temporal average intensity at the point in the ultrasonic field where the temporal average intensity is a maximum (i.e., the spatial peak temporal average intensity) to the spatial average temporal average intensity in a specified plane.

9. Center frequency (f_c) - means $(f_1 + f_2)/2$, where f_1 and f_2 are frequencies defined in bandwidth.

10. Continuous waveform - means a waveform in which the amplitude modulation factor is less than or equal to 5 percent.

11. Depth of focus - means the distance along the beam axis, for a focusing transducer, from the point where the beam cross sectional area first becomes equal to 4 times the focal area to the point beyond the focal surface where the beam cross sectional area again becomes equal to 4 times the focal area.

12. Duty factor - means the product of the pulse duration and the pulse repetition frequency or the ratio of the pulse duration to the pulse repetition period.

13. Envelope - means a waveform which is zero when the instantaneous acoustic pressure and its time derivatives are zero and which otherwise connects the relative maxima on the absolute value of the instantaneous acoustic pressure waveform. The curve connecting these relative maxima should be a cubic natural spline, as defined in IEC Publications 469-1 and 469-2, "Pulse Techniques and Apparatus."

14. Focal area - means the area of the focal surface.

15. Focal length - means the distance along the beam axis from the centroid of the radiating cross sectional area to the focal surface.

16. Focal surface - means the beam cross section with the smallest area of a focusing transducer.

17. Focusing transducer - means a transducer assembly in which the ratio of the smallest beam cross sectional area to the radiating cross sectional area is less than one-fourth.

18. Fractional bandwidth - means bandwidth divided by center frequency.

19. Intensity - means the instantaneous acoustic power transmitted in the direction of acoustic wave propagation, per unit area normal to this direction, at the point considered.

For measurement purposes, this point is restricted to where it is reasonable to assume that the acoustic pressure and particle velocity are in phase; viz, in the far-field or the area near the focal surface. Under such conditions, the intensity can be expressed as

$$I = p^2/\rho c$$

where p is the instantaneous acoustic pressure, ρ is the density of the medium, and c is the velocity of sound in the medium. (Note: the cross sectional measuring area at the point in space must be as small as practicable, generally no larger than $(c/f_c)^2$, where f_c is the center frequency, with the technique and dimensions specified.)

(Note: See also definitions below for pulse average, temporal peak, spatial average-temporal average, spatial peak-pulse average, spatial peak-temporal average, and spatial peak-temporal peak intensities.)

20. Narrowband transducer - means a transducer with a fractional bandwidth less than 15 percent.

21. Pulse average intensity - means the ratio of the pulse intensity integral to the pulse duration or the ratio of the temporal average intensity to the duty factor.

22. Pulse duration - means a time interval beginning when the envelope first exceeds its minimum value plus X percent of the difference between its maximum and minimum values and ending at the last time the envelope returns to this value. For waveforms from therapy equipment X is usually 10 percent; for waveforms from diagnostic equipment X may be larger, for example 32 percent (i.e., minus 10 dB).

In the AIUM/NEMA Standard pulse duration is defined as follows: pulse duration means 1.25 times the interval between the time when the time integral of intensity in an acoustic pulse at a point reaches 10 percent and 90 percent of its final value. The final value of the time integral of intensity is the pulse intensity integral.

23. Pulse intensity integral - means the time integral of intensity, for any specific point and pulse, integrated over the time in which the envelope of acoustic pressure or hydrophone signal for the specific pulse is non-zero. For a transducer assembly operating in a non-auto-scanning mode, this is equivalent to the product of temporal average intensity and pulse repetition period.

24. Pulse repetition period - means the time between corresponding parts in the waveform of successive pulses. The pulse repetition period is equal to the reciprocal of the pulse repetition frequency.

25. Radiating cross sectional area - means the beam cross sectional area at the surface of the transducer assembly. In the AIUM/NEMA Standard radiating cross sectional area is defined as follows: Radiating cross sectional area means the area at and parallel to the transducer face consisting of all points where the acoustic pressure is greater than 10 percent of the maximum acoustic pressure in that plane. The area of the active element of the transducer assembly may be taken as an approximation for the radiating cross sectional area.

26. Spatial average-temporal average intensity (SATA) means the temporal average intensity averaged over the beam cross sectional area in a specified plane (may be approximated as the ratio of ultrasonic power to the beam cross sectional area).

27. Spatial peak-pulse average (SPPA) intensity - means the value of the pulse average intensity at the point in the acoustic field where the pulse average intensity is a maximum, or is a local maximum within a specified region. (May be calculated as the ratio of the spatial peak-temporal average intensity to the duty factor at that point in space.)

28. Spatial peak-temporal average (SPTA) intensity - means the value of the temporal average intensity at the point in the acoustic field where the temporal average intensity is a maximum, or is a local maximum within a specified region.

29. Spatial peak-temporal peak (SPTP) intensity - means the value of the temporal peak intensity at the point in the acoustic field where the temporal peak intensity is a maximum, or is a local maximum within a specified region.

30. Temporal average intensity - means the time average of intensity at a point in space. For non-auto-scanning systems, the average is taken over one or more pulse repetition periods. For auto-scanning systems, the intensity may be averaged over one or more scan repetition periods for a specified operating mode.

31. Temporal peak intensity - means the peak instantaneous value of the intensity at the point considered.

32. Ultrasonic power - usually means the temporal-average power emitted in the form of ultrasonic radiation by a transducer assembly.

In the U.S. Food and Drug Administration's Radiation Safety Performance Standard for Ultrasonic Therapy Products, ultrasonic power is defined as "the total power emitted in the form of ultrasonic radiation by the applicator averaged over each cycle of the ultrasonic radiation carrier wave." This defines power as a function that varies with time. This was so defined because the temporal maximum ultrasonic power is an important quantity in therapeutic applications.

APPENDIX III

The following are comments prepared by the American Institute of Ultrasound in Medicine, Bioeffects Committee regarding the AIUM statement quoted in section 6.1 (AIUM 1978).

"This Statement apparently applies to all existing data on biological changes produced in mammalian tissues by ultrasound in the frequency range from about 0.5 to 10 MHz. Included in our literature review leading to this Statement are results obtained with focussed as well as unfocussed ultrasonic fields, generated continuously or (to a lesser extent) in a series of repeated pulses.

"This Statement included all seemingly reliable data considered in published summaries as well as results of satisfactory quality which have been published more recently. We have consulted a "jury" of informed investigators and have not learned of any exception to the Statement. However, in any application of the Statement to "safety" decisions attention should be given to the following considerations:

"1. Most of the data apply to mammals other than man, and it is not clear how to relate them to the human situation.

"2. While useful results are now being generated in several research laboratories, the pool of reliable and highly relevant data is only beginning to fill. Most experiments have not been repeated by independent investigators. Especially in short supply are results at low intensities and long exposure times. Little research has been done with repeated short pulses such as would be most relevant to diagnostic ultrasound.

"3. Data available at present on intensity levels at which bioeffects occur are, in general, not minimum levels (if, indeed, definite minima exist). Further research is urgently needed to determine whether significant biological changes occur at levels lower than those corresponding to the Statement. As more results become available, it is reasonable to expect at least some lowering of the observed "threshold" levels for some biological systems, especially as more sensitive biological tests are used, and as more critical physical conditions are identified.

"4. We believe the Statement will be helpful in arriving at recommendations for wise use of ultrasound in medicine. However, the Statement does not, in itself, imply specific advice on "safe levels" which might be universally valid. Determination of recommended maximum levels will require consideration of such difficult topics as: adequacy of present knowledge of bioeffects; expected reliability of equipment specifications; assessment of patient benefits; and others. So far these matters have not been treated systematically."

Reference for Appendix III

AIUM Bioeffects Committee. Report of the AIUM Bioeffects Committee. Reflections 4:330 (1978).

APPENDIX IV

Recommendations by AIUM and NEMA

For comparative purposes with the recommendations in this document, statements or attitudes toward ultrasound prepared by the American Institute of Ultrasound in Medicine, Bioeffects Committee (1978) and the general guidelines in the AIUM/NEMA Standard (1981) are quoted below:

AIUM Bioeffects Committee Comments:

"1. In choosing equipment, look for specifications on the output power and intensity. This information has been only rarely available in the past. However, in the future we can expect responsible manufacturers to supply data on these quantities, including the SATA and SPTA intensities.

"2. In comparing equipment, give serious consideration to the intensity levels employed, along with imaging quality, convenience, cost and other factors. Of course, there is no simple formula for arriving at a choice of equipment since there are many variables. However, if two comparable items of equipment seem equally satisfactory in other respects it seems obvious that you should choose the equipment which operates at the lowest intensity.

"3. In choosing procedures and equipment the obligation is always present of balancing benefit against risk. In assessing risk one should not be over simplistic. The SPTA level of 100 mW/cm^2 is not a "magic number" such that (i) one should never use SPTA intensities above this level or such that (ii) one should feel "perfectly safe" in unlimited use of intensities below this level. On the one hand, if there is need for imaging in some medical situation which requires a higher intensity, it may very well be appropriate to use it. On the other hand, ultrasound should certainly not be regarded as a "toy"; it should be used only when a medical benefit is expected. The length of exposure should never be more than needed for the benefit.

"4. One should not be hesitant to use ultrasound, with appropriate equipment and procedures, when benefit is expected. Diagnostic ultrasound has proven itself to be a powerful tool in medical practice. We can take considerable satisfaction in the fact that after years of accumulated experience in clinical use of diagnostic ultrasound there is no known instance of human injury caused by it. This is, indeed, an excellent safety record."

The following represent general guidelines applicable to all diagnostic ultrasound equipment; taken from the AIUM/NEMA draft standard (1981).

"1. Ultrasound equipment should be designed so that the maximum levels of the various intensities which the equipment can produce are as low as practicable for the anticipated uses of the equipment.

"2. Where such flexibility is consistent with reasonable cost and performance of the system, operators should be able to adjust controls to use the minimum acoustical exposure required to image the desired organs on each patient.

"3. As a matter of prudence, users of the equipment should be encouraged by the manufacturer to minimize the acoustical exposure to the patient within the limits of obtaining necessary diagnostic information."

References for Appendix IV

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AIUM/NEMA Standard for Diagnostic Ultrasound Equipment. Publication No. ULI-1981 (1981).

*U.S. GOVERNMENT PRINTING OFFICE : 1982 0-361-177/228

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FDA 81-8162 Handbook of Computed Tomography -X-Ray Spectra (PB 81-295890, $12.00).

FDA 81-8163 Airborne Ultrasound: Measurement and Possible Adverse Effects (GPO 017-015-00193-8, $2.00) (PB 81-240459, mf only).

FDA 81-8164 Calculation Programs for Routine Compliance Testing of Diagnostic X-Ray Systems (PB 81-200503, $9.00).

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